T cell responses to *Chlamydia trachomatis*

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Chlamydia trachomatis is the most common cause of bacterial sexually transmitted disease in the United States, as well as the leading cause of preventable blindness worldwide. Immunity to *C. trachomatis* requires a variety of cell types, each employing an array of effector functions. Recent work has demonstrated that both CD4+ and CD8+ T lymphocytes play a major role in protective immunity to *C. trachomatis*, predominantly through their secretion of interferon-γ. This review describes the generation of acquired immunity to *C. trachomatis* and focuses on how T cells contribute to both protection and immunopathology.

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Abbreviations

APC	antigen-presenting cell
IDO	indoleamine 2,3-dioxygenase
IFN-γ	interferon-γ
MHC	major histocompatibility complex
MOMP	major outer-membrane protein
NO	nitric oxide

Introduction

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that infects the epithelial cells of the genital tract and conjunctivae. It can cause diseases that range from salpingitis and conjunctivitis to pelvic inflammatory disease, the blinding disease trachoma and the systemic infection lymphogranuloma venereum [1]. *Chlamydia* species have a unique lifestyle that is characterized by two distinct developmental forms. The infectious, metabolically inert elementary body (EB) survives outside the host cell, whereas the intracellular, non-infectious reticulate body (RB) replicates within a specialized vacuole called an inclusion. Intracellular replication necessitates an intimate association between the bacterium and the host cell that likely involves altering host cell functions in order to establish and maintain an environment conducive to replication within the inclusion. In addition, given that the compact *Chlamydia* genome lacks many essential biosynthesis genes, the bacteria must scavenge most of their nutrients, including ATP, from the host cell [2].

The intracellular lifestyle of *Chlamydia* undoubtedly influences which elements of host immunity will be effective in reducing the burden of organisms in the host. Although this review focuses on acquired immunity to *C. trachomatis* and the mechanisms by which T cells control the growth of *Chlamydia*, multiple immune mechanisms, including innate responses, are probably employed to control the growth and spread of this organism during infection. Innate resistance to *Chlamydia*, in both human populations and in inbred strains of mice with differential susceptibility to infection, is an exciting area of current investigation [3–6]. Recruitment of inflammatory cells, such as macrophages, to the site of infection and the subsequent release of pro-inflammatory cytokines appear to be crucial for innate resistance to *Chlamydia*.

Acquired immunity to *C. trachomatis*

One approach to identifying the elements of acquired immunity important in resistance to *C. trachomatis* is to observe how the immune system responds to natural infection. However, immunity after a natural infection is not completely effective, as previous exposure to *C. trachomatis* offers only limited protection against re-infection. Most of this protection is serovar-specific and can be attributed largely to antibody specific for the major outer-membrane protein (MOMP), the primary determinant that defines a serovar [7]. Besides studying natural immunity, another equally important approach is to develop immunization strategies that prime the arms of the immune response that are most effective at reducing the replicative capacity of the organism, whether or not these responses are dominant during natural infection.

Current research on acquired immunity to *C. trachomatis* has focused largely on the central role of T lymphocytes in orchestrating the multiple immune mechanisms required to achieve protective immunity. Several steps are required in order for *C. trachomatis* to successfully establish infection, to replicate and to spread. Each stage presents an opportunity for one or more elements of the immune system to block unrestricted growth of the organism. Immediately after re-introduction of *C. trachomatis* into an immune host, it is likely that mucosal and circulating antibodies are able to bind and neutralize some organisms by blocking the ability of these infecting EBs to enter the columnar epithelial cells of the genital tract or conjunctiva. In addition, professional antigen-presenting cells (APCs) present at the site of infection can engulf the organisms and present *Chlamydia*-derived peptide antigens in the context of class II molecules encoded in the major histocompatibility complex (MHC). *Chlamydia*-specific CD4+ T cells are activated after recognition of these peptide–MHC-class-II complexes. As described below, activated CD4+ T cells can both directly inhibit *C. trachomatis* replication as well as stimulate the protective function of a variety of other immune and inflammatory cells. However, once organisms successfully enter epithelial cells, which typically do not express MHC class II, they are able to replicate in a niche in which recognition by CD4+ T cells is unlikely. In epithelial cells, immune surveillance is possible through

recognition of *C. trachomatis* antigens presented in the context of MHC class I molecules to CD8+ T cells. Like CD4+ T cells, CD8+ T cells possess a variety of effector functions capable of limiting *Chlamydia* replication.

Chlamydia-specific CD4+ and CD8+ T cells exert protective effects through secretion of interferon-γ

There is significant data demonstrating that both CD4+ and CD8+ T cells are involved in controlling *C. trachomatis* infection. In human and animal models, both T cell subsets are detected at the site of *C. trachomatis* infection [8–10]. In addition, adoptive transfer of either immune splenocytes or *C. trachomatis*-specific CD4+ or CD8+ T cells into infected mice has been shown to contribute to protection [11–16]. These transferred CD4+ and CD8+ T cells limit *C. trachomatis* infection through a variety of effector mechanisms.

A major function of CD4+ helper T cells is to promote the activation of B cells, CD8+ T cells, and other inflammatory cells, both by contact-dependent and cytokine-mediated processes. A synergistic co-operation between CD4+ T cells and B cells is suggested by a recent study performed by Morrison *et al*. [17•]. The authors showed that μMT mice, which lack B cells, and mice depleted of CD4+ T cells were each slightly more susceptible to a secondary *C. trachomatis* genital tract infection than were wild-type mice. On the other hand, μMT mice that were additionally depleted of CD4+ T cells were severely impaired in their ability to resolve the infection. This study and others [18,19] support a role for neutralizing antibodies in controlling *Chlamydia* infection, and suggest that CD4+ T cells are important regulators of B cell activity.

CD4+ T cells are also likely to be required for optimal stimulation of *C. trachomatis*-specific CD8+ T cells. The CD8+ T cells, in turn, can respond both by secreting interferon-γ (IFN-γ) and by directly lysing infected cells. It has been attractive to speculate that CD8+ T-cell-mediated lysis of infected cells during replication disrupts the developmental cycle of *Chlamydia* and deprives the organism of its replicative niche. However, no evidence to support this hypothesis has been uncovered [20].

Although there continues to be debate about the relative importance of effector mechanisms such as B cell activation and lysis of infected cells, it has consistently been demonstrated that IFN-γ production by CD4+ and CD8+ T cells is critical for protection. Several groups have shown that IFN-γ inhibits the growth of *Chlamydia* in cell culture [21,22]. In addition, disruption of the IFN-γ gene and monoclonal-antibody-mediated neutralization of IFN-γ have both been shown to enhance host susceptibility to *Chlamydia* infection [23–26]. Work in our laboratory suggests that the key mechanism through which CD8+ T cells control *Chlamydia* replication is IFN-γ secretion [15,24]. This work has shown that although adoptive transfer of a *Chlamydia*-specific CD8+ T cell line into naïve mice can mediate protection from challenge, this protective effect cannot be demonstrated when CD8+ T cells are isolated from mice lacking the gene for IFN-γ.

IFN-γ has a variety of activities that appear to limit *C. trachomatis* infection. IFN-γ can upregulate macrophage phagocytic potential and the expression of MHC molecules by both professional and non-professional APCs, leading to enhanced presentation of microbial antigens to both CD4+ and CD8+ T cells [27]. However, these inhibitory activities may be balanced by the ability of *C. trachomatis* to interfere with the IFN-γ-mediated increase in MHC expression. Zhong *et al*. [28] have identified a protease-like factor that is expressed by *Chlamydia* and secreted into the host cell cytosol. This factor has been shown to be both necessary and sufficient for the degradation of host transcription factors required for MHC gene activation.

IFN-γ can also directly inhibit the replication of *C. trachomatis* within infected cells by at least three mechanisms. First, IFN-γ induces the expression of indoleamine 2,3-dioxygenase (IDO), a host enzyme that degrades intracellular tryptophan stores [29,30]. Because *Chlamydia* has little or no ability to produce its own tryptophan, IFN-γ-induced expression of IDO can limit the growth of intracellular *Chlamydia*. Second, IFN-γ upregulates the expression of inducible nitric oxide synthase (iNOS), which catalyzes the production of various reactive nitrogen intermediates, most notably nitric oxide (NO) [31,32]. NO has been identified as an important defense molecule against bacterial pathogens and has been shown to restrict the growth of *Chlamydia in vitro* [33,34•,35]. Third, IFN-γ downregulates the expression of transferrin receptor on the surface of infected cells, resulting in an intracellular iron deficiency that may also limit *C. trachomatis* replication [36]. The relative contributions of the IDO, iNOS and Fe systems in the IFN-γ-mediated control of *Chlamydia* have been found to vary depending on whether the host cells are of human or murine origin. Igietseme *et al*. [33] have shown that each of these IFN-γ-mediated antimicrobial activities contribute, to varying degrees, to the inhibition of intracellular growth of *C. trachomatis* in a human epithelial cell line. NO induction, along with tryptophan and iron deprivation, combined to account for greater than 60% of the IFN-γ-induced growth inhibition in human cells. Induction of IDO in response to *Chlamydia* infection has not been observed in murine systems, but several recent studies show that NO induction plays a role in controlling *Chlamydia* infections in mice [34•,35]. It seems likely that control of intracellular *C. trachomatis* replication will require multiple IFN-γ-dependent inhibitory activities, including some that remain to be identified.

The elements of the immune system most effective at limiting *C. trachomatis* replication probably also promote immunopathology

As described above, it is likely that IFN-γ-mediated activities are able to inhibit the growth of *Chlamydia* in infected

individuals. However, experiments in humans and animals have shown that these activities often result in incomplete clearance of *C. trachomatis* [21,34,37]. The inflammatory response that results from localized IFN-γ production is almost certainly to blame for many of the serious sequelae of *C. trachomatis* infection, such as tissue scarring. IFN-γ production by T cells at the site of infection is likely to diminish as the *Chlamydia* antigen level decreases. Once the T cell response and IFN-γ production wane, *C. trachomatis* is able to resume replication and the inflammatory process repeats. These chronic bouts of conjunctivitis or salpingitis are key to the development of blindness or infertility [38]. Only by stimulating an immune response that sterilizes the site of infection will immune interventions avoid the promotion of immunopathology.

Recognition of *Chlamydia* antigens by T cells depends on type of cell infected and compartmentalization of antigen

Dendritic cells are very efficient APCs that act as sentinels by internalizing pathogens, processing the pathogen-derived proteins into peptide fragments, and then migrating to secondary lymphoid organs, where they present these antigenic peptides to $CD4^+$ and $CD8^+$ T cells [39]. In fact, Shaw *et al.* [40••] recently showed that adoptive transfer of dendritic cells pulsed with non-viable *Chlamydia* into naïve mice afforded significant protection against subsequent challenge with viable *Chlamydia*. They also showed that *Chlamydia*-pulsed dendritic cells secrete a large number of pro-inflammatory molecules that promote their homing to lymphatic tissues and the activation of $CD4^+$ T cells [40••]. These results suggest that dendritic cells are able to efficiently present antigen to T cells and that the resulting activated T cells are protective. Several *Chlamydia*-specific $CD4^+$ T cell lines have been cultured from infected individuals and have been shown to recognize highly expressed *Chlamydia* proteins, such as heat shock protein 60 (Hsp60) and the cell-surface proteins MOMP, Omp2 and PmpD [41–43,44•,45•]. Enolase and open reading frame CT579 have also been identified as $CD4^+$ T cell antigens [45•].

$CD4^+$ T cell activation is an effective mechanism for controlling *Chlamydia* infection in APCs. However, the primary tissue tropism for *Chlamydia* is epithelial cells, which do not typically express MHC class II. Epithelial cells do express MHC class I. Therefore, $CD8^+$ T cells are likely to be important in immune surveillance once the organisms have established infection of the epithelium. $CD8^+$ T cells generally recognize proteins present in the host cell cytosol or cytosolic domains of membrane proteins. These cytosolic proteins are degraded into peptides by the host proteasome, the peptides are transported into the endoplasmic reticulum, where they bind the MHC class I molecules, and the peptide–MHC-class-I complexes are transported to the cell surface. Even though *Chlamydia* remain confined to a vacuolar compartment throughout their replication cycle, $CD8^+$ T cells are able to recognize *Chlamydia*-infected cells *in vitro* and *in vivo* [15,16,46,47]. This suggests that one or more *Chlamydia* protein has access to the host cell cytosol. Among the classes of proteins that may have access to the host cell cytoplasm are several inclusion membrane proteins as well as putative substrates of a *Chlamydia* type III secretion system. Type III secretion systems are envelope-associated protein complexes capable of directly translocating proteins from the bacterial cytoplasm into the host cell cytosol. This type of secretion apparatus has been identified in a broad range of bacteria [48]. Sequence analysis of the *Chlamydia* genome has identified a potential type III secretion system that may be able to translocate *C. trachomatis* proteins across the inclusion membrane into the host cell cytosol [2,49]. It is not known which *Chlamydia* proteins, if any, are secreted by this system. However, some secreted *Chlamydia* proteins are likely to act as $CD8^+$ T cell antigens. Thus far, only a few $CD8^+$ T cell antigens have been identified in *Chlamydia*. *Chlamydia*-specific $CD8^+$ T cell lines isolated from humans have been shown to recognize peptide fragments derived from MOMP [50•]. A well-characterized $CD8^+$ T cell line cultured from mice is specific for Cap1, a *C. trachomatis* protein of unknown function that localizes to the inclusion membrane [51•]. As more $CD8^+$ T cell antigens are identified, they can be incorporated into vaccine delivery vehicles with antigens that stimulate $CD4^+$ T cells and can be tested for their protective ability. Because recognition of $CD8^+$ T cell antigens suggests cytosolic or inclusion membrane localization, these proteins may be involved in functions essential for the intracellular survival of *C. trachomatis*. Such functions may include scavenging of nutrients or modification of host cell structures or processes. In addition to their use in vaccines, characterization of these proteins may suggest non-immune therapeutic interventions capable of inhibiting *C. trachomatis*.

Conclusions: both $CD4^+$ and $CD8^+$ T cell responses are required for optimal immunity to *C. trachomatis*

In many of their functions, $CD4^+$ and $CD8^+$ T cells are interdependent and redundant. Therefore, it is difficult to dissect the relative contributions of one subset by eliminating the other. This is particularly true when one considers that undermining the activity of $CD4^+$ T cells may disrupt the activity of several other cell types. Selective depletion of either $CD4^+$ or $CD8^+$ T cells in experimental mice reduces the capacity of the animals to resolve *C. trachomatis* infection [17•,52]. Several investigators have found that, in animals depleted of $CD4^+$ T cells or in MHC class II knockout animals, the effect on protection is more dramatic than in animals depleted of $CD8^+$ T cells or in β2-microglobulin knockout mice [53,54]. Some have used these data to argue that $CD8^+$ T cells are not as important as $CD4^+$ T cells in immunity to *C. trachomatis* [53,54]. However, such an interpretation is complicated in part because $CD4^+$ T cells are required for optimal stimulation of $CD8^+$ T cells. A similar interdependence can be argued when considering the role of B cells in *Chlamydia*-specific

immunity, given that elimination of CD4+ T cells would also impair B cell stimulation and secretion of specific antibody. The redundancy of the immune response to *C. trachomatis* is revealed in the finding that both CD4+ and CD8+ T cells exert their protective effects primarily through the secretion of IFN-γ. As a result, an ideal immunization strategy may simply require stimulation of a critical threshold frequency of IFN-γ-secreting T cells in order for optimal protection to be achieved.

In studies with model viral pathogens, it has been possible to provide sterilizing protective immunity by stimulating T cells with a single peptide reactivity [55]. This has not held true when attempting to design vaccines against bacterial pathogens. It is almost certain that vaccines designed to stimulate protective immunity against *C. trachomatis* and other intracellular bacterial pathogens will require the incorporation of several antigens. An effective mix of antigens would include those able to stimulate multiple arms of the immune system, including CD4+ T cells, CD8+ T cells and antibody production.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Grayston JT, Wang S: **New knowledge of chlamydiae and the diseases they cause.** *J Infect Dis* 1975, **132**:87-105.
2. Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, Mitchell W, Olinger L, Tatusov RL, Zhao Q *et al.*: **Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*.** *Science* 1998, **282**:754-759.
3. Mozzato-Chamay N, Mahdi OS, Jallow O, Mabey DC, Bailey RL, Conway DJ: **Polymorphisms in candidate genes and risk of scarring trachoma in a *Chlamydia trachomatis*-endemic population.** *J Infect Dis* 2000, **182**:1545-1548.
4. Monno R, Vena G, Cafforio P, Milone E: **Polymorphonuclear cell function impairment in patients with *Chlamydia trachomatis* urogenital infections.** *Acta Microbiol Hung* 1991, **38**:75-79.
5. Darville T, Andrews CW Jr, Sikes JD, Fraley PL, Rank RG: **Early local cytokine profiles in strains of mice with different outcomes from chlamydial genital tract infection.** *Infect Immun* 2001, **69**:3556-3561.
6. Stagg AJ, Tuffrey M, Woods C, Wunderink E, Knight SC: **Protection against ascending infection of the genital tract by *Chlamydia trachomatis* is associated with recruitment of major histocompatibility complex class II antigen-presenting cells into uterine tissue.** *Infect Immun* 1998, **66**:3535-3544.
7. Brunham RC: **Human immunity to chlamydiae.** In *Chlamydia: Intracellular Biology, Pathogenesis, and Immunity*. Edited by Stephens RS. Washington, DC: ASM Press; 1999:211-238.
8. Van Voorhis WC, Barrett LK, Sweeney YT, Kuo CC, Patton DL: **Analysis of lymphocyte phenotype and cytokine activity in the inflammatory infiltrates of the upper genital tract of female macaques infected with *Chlamydia trachomatis*.** *J Infect Dis* 1996, **174**:647-650.
9. Kelly KA, Walker JC, Jameel SH, Gray HL, Rank RG: **Differential regulation of CD4 lymphocyte recruitment between the upper and lower regions of the genital tract during *Chlamydia trachomatis* infection.** *Infect Immun* 2000, **68**:1519-1528.
10. Johansson M, Lycke N: **Immunological memory in B-cell-deficient mice conveys long-lasting protection against genital tract infection with *Chlamydia trachomatis* by rapid recruitment of T cells.** *Immunology* 2001, **102**:199-208.
11. Brunham RC, Kuo C, Chen WJ: **Systemic *Chlamydia trachomatis* infection in mice: a comparison of lymphogranuloma venereum and trachoma biovars.** *Infect Immun* 1985, **48**:78-82.
12. Zhong G, Peterson EM, Czarniecki CW, Schreiber RD, de la Maza LM: **Role of endogenous gamma interferon in host defense against *Chlamydia trachomatis* infections.** *Infect Immun* 1989, **57**:152-157.
13. Su H, Caldwell HD: **CD4+ T cells play a significant role in adoptive immunity to *Chlamydia trachomatis* infection of the mouse genital tract.** *Infect Immun* 1995, **63**:3302-3308.
14. Thoma-Uszynski S, Simnacher U, Marre R, Essig A: **Clearance of *Chlamydia trachomatis*-induced polyserositis in SCID mice requires both CD4+ and CD8+ cells.** *Med Microbiol Immunol (Berl)* 1998, **187**:71-78.
15. Starnbach MN, Bevan MJ, Lampe MF: **Protective cytotoxic T lymphocytes are induced during murine infection with *Chlamydia trachomatis*.** *J Immunol* 1994, **153**:5183-5189.
16. Igietseme JU, Magee DM, Williams DM, Rank RG: **Role for CD8+ T cells in antichlamydial immunity defined by *Chlamydia*-specific T-lymphocyte clones.** *Infect Immun* 1994, **62**:5195-5197.
17. • Morrison SG, Su H, Caldwell HD, Morrison RP: **Immunity to murine *Chlamydia trachomatis* genital tract reinfection involves B cells and CD4(+) T cells but not CD8(+) T cells.** *Infect Immun* 2000, **68**:6979-6987.

Using *in vivo* antibody depletion of CD4+ and CD8+ T cells in wild-type mice and μMT mice, which lack B cells, the authors showed that CD4+-depleted μMT mice are more susceptible to a secondary *Chlamydia* infection. This suggests that there is a synergistic interaction between B cells and CD4+ T cells. Depletion of CD8+ T cells appeared to have no effect on immunity to *Chlamydia* in either the presence or absence of B cells.

18. Yang X, Brunham RC: **Gene knockout B cell-deficient mice demonstrate that B cells play an important role in the initiation of T cell responses to *Chlamydia trachomatis* (mouse pneumonitis) lung infection.** *J Immunol* 1998, **161**:1439-1446.
19. Su H, Feilzer K, Caldwell HD, Morrison RP: ***Chlamydia trachomatis* genital tract infection of antibody-deficient gene knockout mice.** *Infect Immun* 1997, **65**:1993-1999.
20. Perry LL, Feilzer K, Hughes S, Caldwell HD: **Clearance of *Chlamydia trachomatis* from the murine genital mucosa does not require perforin-mediated cytolysis or Fas-mediated apoptosis.** *Infect Immun* 1999, **67**:1379-1385.
21. Shemer Y, Sarov I: **Inhibition of growth of *Chlamydia trachomatis* by human gamma interferon.** *Infect Immun* 1985, **48**:592-596.
22. Kane CD, Vena RM, Ouellette SP, Byrne GI: **Intracellular tryptophan pool sizes may account for differences in gamma interferon-mediated inhibition and persistence of chlamydial growth in polarized and nonpolarized cells.** *Infect Immun* 1999, **67**:1666-1671.
23. Perry LL, Su H, Feilzer K, Messer R, Hughes S, Whitmire W, Caldwell HD: **Differential sensitivity of distinct *Chlamydia trachomatis* isolates to IFN-gamma-mediated inhibition.** *J Immunol* 1999, **162**:3541-3548.
24. Lampe MF, Wilson CB, Bevan MJ, Starnbach MN: **Gamma interferon production by cytotoxic T lymphocytes is required for resolution of *Chlamydia trachomatis* infection.** *Infect Immun* 1998, **66**:5457-5461.
25. Johansson M, Schon K, Ward M, Lycke N: **Studies in knockout mice reveal that anti-chlamydial protection requires TH1 cells producing IFN-gamma: is this true for humans?** *Scand J Immunol* 1997, **46**:546-552.
26. Rank RG, Ramsey KH, Pack EA, Williams DM: **Effect of gamma interferon on resolution of murine chlamydial genital infection.** *Infect Immun* 1992, **60**:4427-4429.
27. Martin-Orozco N, Isibasi A, Ortiz-Navarrete V: **Macrophages present exogenous antigens by class I major histocompatibility complex molecules via a secretory pathway as a consequence of interferon-gamma activation.** *Immunology* 2001, **103**:41-48.
28. Zhong G, Fan P, Ji H, Dong F, Huang Y: **Identification of a chlamydial protease-like activity factor responsible for the degradation of host transcription factors.** *J Exp Med* 2001, **193**:935-942.

29. Gupta SL, Carlin JM, Pyati P, Dai W, Pfefferkorn ER, Murphy MJ Jr: **Antiparasitic and antiproliferative effects of indoleamine 2,3-dioxygenase enzyme expression in human fibroblasts.** *Infect Immun* 1994, **62**:2277-2284.

30. Murray HW, Szuro-Sudol A, Wellner D, Oca MJ, Granger AM, Libby DM, Rothermel CD, Rubin BY: **Role of tryptophan degradation in respiratory burst-independent antimicrobial activity of gamma interferon-stimulated human macrophages.** *Infect Immun* 1989, **57**:845-849.

31. Liew FY, Cox FE: **Nonspecific defence mechanism: the role of nitric oxide.** *Immunol Today* 1991, **12**:A17-A21.

32. Liew FY: **Regulation of nitric oxide synthesis in infectious and autoimmune diseases.** *Immunol Lett* 1994, **43**:95-98.

33. Igietseme JU, Ananaba GA, Candal DH, Lyn D, Black CM: **Immune control of chlamydial growth in the human epithelial cell line RT4 involves multiple mechanisms that include nitric oxide induction, tryptophan catabolism and iron deprivation.** *Microbiol Immunol* 1998, **42**:617-625.

34. • Ramsey KH, Miranpuri GS, Sigar IM, Ouellette S, Byrne GI: ***Chlamydia trachomatis* persistence in the female mouse genital tract: inducible nitric oxide synthase and infection outcome.** *Infect Immun* 2001, **69**:5131-5137.
The authors of this paper compared the ability of IFN-γ-treated iNOS$^{+/+}$ and iNOS$^{-/-}$ fibroblasts to control the growth of *Chlamydia in vitro* and found that growth inhibition occurred in both cell types, but was reversible after removal of IFN-γ in the iNOS$^{-/-}$ cell culture only. These results suggested that additional IFN-γ-induced mechanisms contribute to the eradication of *Chlamydia*, but that these mechanisms are simply bacteristatic in the absence of iNOS activity.

35. Chen B, Stout R, Campbell WF: **Nitric oxide production: a mechanism of *Chlamydia trachomatis* inhibition in interferon-gamma-treated RAW264.7 cells.** *FEMS Immunol Med Microbiol* 1996, **14**:109-120.

36. Byrd TF, Horwitz MA: **Regulation of transferrin receptor expression and ferritin content in human mononuclear phagocytes. Coordinate upregulation by iron transferrin and downregulation by interferon gamma.** *J Clin Invest* 1993, **91**:969-976.

37. Bobo LD, Novak N, Munoz B, Hsieh YH, Quinn TC, West S: **Severe disease in children with trachoma is associated with persistent *Chlamydia trachomatis* infection.** *J Infect Dis* 1997, **176**:1524-1530.

38. Kunimoto D, Brunham RC: **Human immune response and *Chlamydia trachomatis* infection.** *Rev Infect Dis* 1985, **7**:665-673.

39. Rescigno M, Borrow P: **The host–pathogen interaction: new themes from dendritic cell biology.** *Cell* 2001, **106**:267-270.

40. •• Shaw JH, Grund VR, Durling L, Caldwell HD: **Expression of genes encoding Th1 cell-activating cytokines and lymphoid homing chemokines by *Chlamydia*-pulsed dendritic cells correlates with protective immunizing efficacy.** *Infect Immun* 2001, **69**:4667-4672.
This work convincingly demonstrates that dendritic cells can serve as potent stimulators of T cell immunity. This suggests that vaccine delivery vehicles that target *C. trachomatis* antigens to dendritic cells may result in greater levels of protection.

41. Deane KH, Jecock RM, Pearce JH, Gaston JS: **Identification and characterization of a DR4-restricted T cell epitope within chlamydia heat shock protein 60.** *Clin Exp Immunol* 1997, **109**:439-445.

42. Kinnunen A, Paavonen J, Surcel HM: **Heat shock protein 60 specific T-cell response in chlamydial infections.** *Scand J Immunol* 2001, **54**:76-81.

43. Su H, Morrison RP, Watkins NG, Caldwell HD: **Identification and characterization of T helper cell epitopes of the major outer membrane protein of *Chlamydia trachomatis*.** *J Exp Med* 1990, **172**:203-212.

44. • Kim S, DeMars R: **Epitope clusters in the major outer membrane protein of *Chlamydia trachomatis*.** *Curr Opin Immunol* 2001, **13**:429-436.
The authors describe overlapping clusters of peptides encoded within MOMP that are recognized by *Chlamydia*-specific CD4$^+$ and CD8$^+$ T cells.

45. • Goodall JC, Yeo G, Huang M, Raggiaschi R, Gaston JS: **Identification of *Chlamydia trachomatis* antigens recognized by human CD4$^+$ T lymphocytes by screening an expression library.** *Eur J Immunol* 2001, **31**:1513-1522.
Using *Chlamydia*-specific T cell clones, the authors identified three novel *C. trachomatis* antigens recognized by CD4$^+$ lymphocytes: the putative membrane protein, PmpD; a glycolytic enzyme called enolase; and CT579, a predicted YopD ortholog.

46. Ojcius DM, Bravo de Alba Y, Kanellopoulos JM, Hawkins RA, Kelly KA, Rank RG, Dautry-Varsat A: **Internalization of *Chlamydia* by dendritic cells and stimulation of *Chlamydia*-specific T cells.** *J Immunol* 1998, **160**:1297-1303.

47. Beatty PR, Stephens RS: **CD8$^+$ T lymphocyte-mediated lysis of *Chlamydia*-infected L cells using an endogenous antigen pathway.** *J Immunol* 1994, **153**:4588-4595.

48. Hueck CJ: **Type III protein secretion systems in bacterial pathogens of animals and plants.** *Microbiol Mol Biol Rev* 1998, **62**:379-433.

49. Kim JF: **Revisiting the chlamydial type III protein secretion system: clues to the origin of type III protein secretion.** *Trends Genet* 2001, **17**:65-69.

50. • Kim SK, Devine L, Angevine M, DeMars R, Kavathas PB: **Direct detection and magnetic isolation of *Chlamydia trachomatis* major outer membrane protein-specific CD8$^+$ CTLs with HLA class I tetramers.** *J Immunol* 2000, **165**:7285-7292.
The authors of this paper identified CD8$^+$ T cell epitopes within MOMP. Using tetramer analysis, they were able to detect MOMP-specific T cells in the peripheral blood of *Chlamydia*-infected humans, suggesting that an HLA-class-I-restricted CD8$^+$ T cell response specific for MOMP is primed during infection.

51. • Fling SP, Sutherland RA, Steele LN, Hess B, D'Orazio SE, Maisonneuve J, Lampe MF, Probst P, Starnbach MN: **CD8$^+$ T cells recognize an inclusion membrane-associated protein from the vacuolar pathogen *Chlamydia trachomatis*.** *Proc Natl Acad Sci USA* 2001, **98**:1160-1165.
This study uses a novel approach to identify CD8$^+$ T-cell antigens from bacterial pathogens. *Chlamydia*-specific CD8$^+$ T-cell lines cultured from infected mice were used to screen expression libraries of *C. trachomatis* genes. This approach resulted in the identification of the Cap1 antigen.

52. Magee DM, Williams DM, Smith JG, Bleicker CA, Grubbs BG, Schachter J, Rank RG: **Role of CD8 T cells in primary *Chlamydia* infection.** *Infect Immun* 1995, **63**:516-521.

53. Morrison RP, Feilzer K, Tumas DB: **Gene knockout mice establish a primary protective role for major histocompatibility complex class II-restricted responses in *Chlamydia trachomatis* genital tract infection.** *Infect Immun* 1995, **63**:4661-4668.

54. Williams DM, Grubbs BG, Pack E, Kelly K, Rank RG: **Humoral and cellular immunity in secondary infection due to murine *Chlamydia trachomatis*.** *Infect Immun* 1997, **65**:2876-2882.

55. Klavinskis LS, Whitton JL, Joly E, Oldstone MB: **Vaccination and protection from a lethal viral infection: identification, incorporation, and use of a cytotoxic T lymphocyte glycoprotein epitope.** *Virology* 1990, **178**:393-400.