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A re-evaluation of the role of B cells in protective immunity to *Chlamydia* infection

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Abstract

Chlamydia trachomatis is the etiological agent of the most commonly reported bacterial sexual transmitted infection (STI) in North America and Europe. The control of *Chlamydia* infection is hindered by the asymptomatic nature of initial infection but the consequence of untreated infection seriously threatens the reproductive health of young women. Unfortunately, there is no licensed vaccine for *Chlamydia* vaccine, in part due to our incomplete understanding of the immune response to *Chlamydia* urogenital infection. It has been well established that T cell-mediated immunity plays a dominant role in protective immunity against *Chlamydia* and thus the importance of B cells is somewhat underappreciated. Here, we summarize recent progress on understanding the role of B cells during *Chlamydia* genital tract infections and discuss how B cells and humoral immunity make an effective contribution to host defense against important intracellular pathogens, including *Chlamydia*.

Keywords

Chlamydia; intracellular infections; B cells; antibody

1. Introduction

Chlamydia trachomatis is a gram-negative, obligate intracellular bacterium that causes the most prevalent sexual transmitted infection (STI) worldwide. According to the Centers for Disease Control (CDC), *Chlamydia* is now the most commonly reported infectious disease in the United States with more than 3 million cases occurring annually [1,2]. Unfortunately most women with urogenital *Chlamydia* experience a subclinical infection, yet these untreated *Chlamydia* infections can lead to severe reproductive problems such as pelvic inflammatory disease (PID), ectopic pregnancy and involuntary infertility, meaning that *Chlamydia* infections represent a growing threat to the reproductive health of young women [3]. Despite the implementation of a *Chlamydia* screening and treatment program in many

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high-income countries over the past decade, the prevalence of *Chlamydia* infection has continued to increase every year [4]. There is now a consensus among the medical and research community that an effective *Chlamydia* vaccine is required [5,6]. However, for this to become a reality, a greater understanding of the mechanisms of *Chlamydia* pathogenesis and the induction of host protective immunity will be required.

Chlamydia is an obligate intracellular pathogen and it is commonly thought that protective immunity to this class of pathogen is largely conferred by an appropriate cell-mediated immune (CMI) response. Indeed, it is generally accepted that CD4 T cells plays a predominant role in protective immunity to *Chlamydia* infection, whereas the requirement for antibody and/or B cells is limited [3,7,8]. Although there is certainly a collection of evidence to support the protective contribution of Th1 cells in a variety of intracellular infections [9–12], the easy assumption that intracellular organisms are outside the reach of the humoral immune responses deserves careful consideration [13]. Indeed, there is emerging evidence to support a prominent role for B cell-mediated immunity in several intracellular infection models, including *Chlamydia*, challenging the paradigm that such pathogens are beyond the reach of the humoral response. In this review, we attempt to highlight the progress made over the past few years to further define the role of antibody and B cells in *Chlamydia* genital tract infection models and also in vaccination studies with this pathogen.

2. Historical paradigms

Before the availability of gene-deficient mice, the role of B cells in *Chlamydia* infection was examined using reagents that suppressed humoral immunity in small animal models. When the humoral immune response was suppressed in Guinea pigs by cyclophosphamide treatment, genital infection with Guinea Pig Inclusion Conjunctivitis (GPIC, also called *Chlamydia caviae*) failed to resolve [14]. Similarly, treatment with cyclophosphamide was able to diminish host protective immunity against secondary *Chlamydia* infection [15]. Consistent with these observations, the passive transfer of immune serum from previously infected animals was able to significantly reduce bacterial shedding from the genital tract of naïve guinea pigs [16]. Conversely, in murine models, the depletion of B cells using anti-IgM antibody suggested no clear role for B cells in the resolution of primary and secondary infection with *Chlamydia muridarum* (a natural mouse pathogen closely related to *Chlamydia trachomatis*) [17]. Despite the discordant findings in these two models, both groups of infected animals developed long-lasting antibody responses reflected by high titers of *Chlamydia*-specific IgGs in the serum [18,19].

When gene-deficient mice with immunological defects became available [20,21], Su *et al* confirmed that the duration and intensity of *Chlamydia muridarum* primary infection was indistinguishable in wild-type and B cell deficient mice (μ MT), as determined by bacterial shedding measured by vaginal swabs [20]. However, in response to a secondary infection with the same pathogen, μ MT mice exhibited a small, but significant, increase in infection susceptibility [20]. These data suggested a minor role for B cells in secondary protective immunity. In marked contrast, numerous studies demonstrated a major role for CMI in the clearance of *Chlamydia* infection. Thus, mice lacking T cells (athymic nude mice, $\text{TCR}\beta^{-/-}$),

or MHC class II-restricted CD4 T cells (MHCII^{-/-} mice), developed chronic *Chlamydia* infection that did not resolve [22–24]. Together, these findings from gene-deficient mice provided support for a conceptual framework that pointed to CMI responses mediating protection against intracellular infections and minimal contribution from B cells and antibody.

3. B cells and *Chlamydia* infection: mouse model revisited

While the studies outlined above support a major role for T cells in *Chlamydia* clearance, they do not completely rule out the possibility that B cells actively participate in bacteria clearance [13]. As noted above, mice lacking B cells show increased susceptibility to secondary infection, indicating some protective role for B cells. Furthermore, the studies of T cell-deficient mice rarely considered the fact these animals also lacked T cell dependent antibody, meaning that increased susceptibility could also reflect a major defect in humoral immunity. In an effort to unravel the contribution of these different arms of adaptive immunity during secondary *Chlamydia* infection, Morrison and colleagues conducted a series of important antibody-depletion experiments in wild-type and B-cell deficient mice. By removing either CD4 or CD8 T cells, or both populations, they were able to demonstrate that when antibody-mediated immunity (AMI) is intact, neither CD4 T cells nor CD8 T cells are absolutely required for the resolution of secondary *Chlamydia* infection. In contrast, when similar experiments were conducted in B cell-deficient mice, these mice were unable to resolve secondary infection in the absence of CD4 T cells [25,26]. Together, these findings suggest a fundamental difference between the mechanisms of protective immunity against primary and secondary *Chlamydia* infection. While the resolution of *Chlamydia* primary infection seems to be dependent on the response of CD4 T cells, secondary protection against *Chlamydia* infection can be conferred by CD4 T cells or B cells. The predominant role of antibody in protective immunity against *Chlamydia* reinfections was further supported by a follow-up study by Morrison and colleagues demonstrating that the passive transfer of immune convalescent serum to B cell-deficient mice conferred full protection to the host against *Chlamydia* reinfection in the absence of CMI [27].

An additional aspect to consider is that there is increasing evidence for B cell effector functions beyond simply producing pathogen-specific antibody [28]. More recently, our laboratory addressed whether B cells can act as antigen presenting cells during a primary host response to *Chlamydia* genital tract infection by revisiting the μ MT mouse model. The issue being examined was whether the lack of B cells in these animals directly affected the clonal expansion of *Chlamydia*-specific CD4 T cells. Using *Chlamydia*-specific MHC class-II tetramers, we demonstrated that the endogenous, antigen-specific CD4 T cell responses to *Chlamydia* infection is significantly reduced within the local draining iliac lymph nodes of B cell-deficient mice. Strikingly, while the kinetics of bacterial shedding from the genital tract remained unaltered by the absence of B cells, μ MT mice displayed a transient disseminated infection in the spleen and peritoneal cavity and developed ascites, a phenotype that actually closely resembles human peritonitis and severe PID associated with *C. trachomatis* genital tract infection [29–32]. Surprisingly, this disseminated infection in μ MT mice correlated with a robust systemic immune response in both the spleen and peritoneal cavity, perhaps representing a compensatory response by the host defending against disseminated bacteria

[33]. The exact mechanism underlying this unexpected requirement for B cells in early primary bacterial containment in the reproductive tract is under active investigation, but these findings reveal an unexpected but indispensable role of B cells in *Chlamydia* primary infection.

4. Protective role of B cells and antibody in *Chlamydia* infection

The mechanism by which B cells and/or antibody protect the host against *Chlamydia* infection is not completely clear. Numerous in vitro studies have documented a definitive role of antibody in *Chlamydia* neutralization and complement activation [34–39]. Indeed, adoptive transfer of monoclonal antibodies against immunodominant epitopes, such as MOMP (major outer membrane protein), can protect mice against *Chlamydia* genital tract infection [37,40]. In contrast, antibodies against other antigens such as hsp60 (heat-shock protein 60) are not protective and some have even been associated with the development of immune-mediated pathology [27,41,42]. An interesting study by Caldwell and colleagues has demonstrated that pre-existing antibodies against major surface antigens such as MOMP and LPS can block the binding of a more protective antibody against PmpD (polymorphic membrane protein D) [43]. Therefore, given the unique intracellular biphasic developmental cycle and temporal regulation of gene expression of *Chlamydia* [44], the contribution of different antibody responses to protection or inhibiting protection adds an additional layer of complexity to the overall antibody response. The importance of complement fixation is also less clear from in vivo studies than historically has been observed in vitro. Nevertheless, recent reports have shed some light on the role of different complement components during *Chlamydia* infection. It has been demonstrated that C3 and C3aR both play a protective role in *Chlamydia psittaci* lung infection, whereas C5 but not C3 contribute to development of immunopathology at the mouse upper genital tract [45–47].

Despite the possibility for direct antibody-dependent neutralization or complement-mediated killing, these mechanisms are unlikely to account for antibody-mediated protection during secondary infection. This conclusion is based on the observation that the passive transfer of immune serum is only able to protect antigen-experienced hosts, rather than naïve animals [44]. Morrison et al, has suggested that this unique feature of the model reflects “CD4 T cell-mediated adaptive changes” that occur in the local genital tract tissue during initial infection, and that these modifications are required for protective antibody to elicit an effector function. This model suggests that interaction of antibody with Fc receptors on non-CD4 T cells is required for antibody-mediated protective immunity. In line with this hypothesis, Moore et al, have reported that *Chlamydia*-specific antibody can enhance antigen presentation of FcR^{+/+} but not FcR^{-/-} antigen presenting cells (APCs) [48,49].

Early infection of the genital mucosa presents a crucial “window of opportunity” for the host to restrict pathogen spread [50]. The disseminated infection during the early phase of primary *Chlamydia* infection of μ MT mice points to an indispensable role of B cells in local pathogen containment [33]. The marked reduction in antigen-specific CD4 T cell priming within the draining iliac lymph nodes could suggest that B cells participate directly in antigen presentation during the earliest stages of primary infection. Although direct evidence from mice lacking MHC class-II expression specifically on B cells would required to

support this idea, additional studies using the *C. muridarum* lung infection model have demonstrated that mice lacking B cells fail to initiate an efficient CD4 T cell response to intranasal infection [51]. Despite the attractiveness of B cells participating as antigen-presenting cells, several alternative possibilities are equally viable (Figure 1). First, as a major component of secondary lymphoid organs (SLOs), B cells are known to be essential for the maintenance of the lymphoid architecture. Thus, the defective *Chlamydia*-specific CD4 T cell response that is observed in μ MT mice could be a consequence of disrupted lymphoid structure leading to inefficient antigen presentation to T cells. Second, a recent study has suggested that B cells are essential for the maintenance of sub-capsular sinus (SCS) macrophages in the draining lymph node. The lack of SCS macrophages explains the inability of μ MT mice to defend against some viral infections [52]. A similar mechanism involving a requirement for SCS macrophages could therefore account for disseminated infection in μ MT mice after *Chlamydia* infection. Third, B cells can elicit effector functions by producing various cytokines in response to microbial stimulation and these cytokines may contribute to local defense in the lymph node that prevents bacterial dissemination. Lastly, a very early antibody response to *Chlamydia* infection may contribute to bacteria containment within the draining lymph node and local tissues. This possibility is supported by the notion that unlike the well-characterized high-affinity antibody that usually developed later during primary infection and can confer protection to reinfections, a rapid extra-follicular B cell response can result in antibody development very early during a primary infection [53]. Further studies are required to determine which of these alternative hypotheses explains the contribution of B cells to preventing bacterial dissemination during primary *Chlamydia* infection.

5. B cells and intracellular infections: lesson learned from others

While the exact mechanism of B cell-mediated protective immunity is currently unclear for *Chlamydia* genital tract infection, some lesson can be learned from the study of B cells in other intracellular pathogen models. For example, it has been established that defined monoclonal antibodies (mAbs) are able to protect the host against *Mycobacterium tuberculosis* (Mtb) infection [54–56]. Moreover, as constituents of ectopic germinal centers in the lung, B cells are able to modulate the inflammatory response during Mtb infection and have recently been shown to regulate host neutrophilia by affecting IL-17 responses [57,58].

The protective role of B cell in *Salmonella* infection has been described as involving antibody-dependent and antibody-independent mechanisms [59–61]. For example, it has been shown that an early isotype-switched antibody response that is established as early as 3 days after *Salmonella* infection is able to impair colonization of *Salmonella* in the spleen and reduce bacteremia [62]. Other studies suggest a regulatory function of B cells during *Salmonella* infection as manifested by B cell-dependent cytokine production that alters the effector function of CD4 T cells to make IFN- γ and IL-17 [63,64]. Lastly, although serum transfers can reduce bacterial loads [60,65], it has been demonstrated that B cell production of antibody is not actually essential to the protective effect of B cells upon the host response [61]. Indeed, Th1 responses are found to be significantly reduced in *Salmonella*-infected B cell-deficient mice [59,61], demonstrating a prominent role for non-antibody-dependent mechanisms in this model.

There is also a growing body of evidence for antibody-mediated protection against other obligate intracellular pathogens. Passive transfer of immune serum protects naïve immune competent hosts from *Coxiella burnetii*, the etiological agent for Q fever [66], although the exact protective mechanism in this model remains unclear since both Fc receptor and complement seem to be dispensable [67]. Interestingly, in an effort to find the protective mechanism mediated by a *C. burnetii* phase I vaccine (PI-V) strain, Zhang et al demonstrated that a switched IgG response to PI-V lipopolysaccharide (LPS) provided significant protection to the host to an extent comparable to PI-V vaccination against *C. burnetii* infection [68]. Similar observations have also been made in obligate intracellular *Ehrlichia chaffeensis* infections, where antibody recognizing the major outer membrane protein (OMP)-1g of *E. chaffeensis* protects SCID mice from ehrlichiosis, a vector-borne disease [69].

How might antibody mediate protection against an obligate intracellular pathogen? The discovery of protective antibody to *E. chaffeensis* infection led Li et al to uncover an extracellular phase in the *Ehrlichia* life cycle [70]. Although this may not be relevant for *Chlamydia*, it does imply that for an obligate intracellular bacterium, even brief exposure outside host cells can make them vulnerable to antibody-mediated defense mechanisms. Alternatively, rather than provide a killing mechanism extra-cellularly, it is possible that some antibodies can find their way inside infected cells. Recent discovery of intracellular Fc receptor, TRIM21 has suggested an intracellular defending mechanism for antibody-mediated protection to viral and bacteria infections [71,72].

6. Concluding remarks

The increasing prevalence of *Chlamydia* STI, the asymptomatic nature of *Chlamydia* infection in young women, and the irreversible disease sequelae, all argue for the development of an effective *Chlamydia* vaccine [5]. While the majority of vaccine studies in the past two decades have focused on using the whole organism or a handful of immunodominant proteins, recent attempts have examined subunit vaccines that are able to elicit robust CD4 T cell responses [73–75]. Nevertheless, even in these vaccine models, the fact that the native conformation of the immune-dominant proteins is essential for protective immunity might suggest that B cells actively participate in the protective immunity conferred by vaccination [76–78]. The murine model of *Chlamydia* genital tract infection has provided an invaluable system for immunologists to understand the host immune response to this infection and as noted above, recent data support a previously unappreciated role of B cells in preventing bacteria dissemination from the genital tract reinfection. Fortunately, with recent advances in proteomics, the screening of large numbers of antigens using serum samples from previously infected mice, non-human primates, and human patients has lead to discovery of a new array of antigen targets in *Chlamydia* [79–81]. Although the understanding of the role of B cells and antibody in *Chlamydia* infection is still in its infancy, the recent progress discussed in this review supports the idea that this population play an important role in the adaptive immune response to infection and thus may be key to the development of a novel vaccine for *Chlamydia*.

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References

1. WHO. Global incidence and prevalence of selected curable sexually transmitted infections. In: Organization WH. , editor. Geneva, Switzerland: 2008.
2. CDC. Atlanta: U.S. Department of Health and Human Services; 2014. Sexually Transmitted Disease Surveillance 2012.
3. Brunham RC, Rey-Ladino J. Immunology of Chlamydia infection: implications for a Chlamydia trachomatis vaccine. *Nat Rev Immunol*. 2005; 5:149–161. [PubMed: 15688042]
4. Rekart ML, Gilbert M, Meza R, Kim PH, Chang M, Money DM, et al. Chlamydia public health programs and the epidemiology of pelvic inflammatory disease and ectopic pregnancy. *J Infect Dis*. 2013; 207:30–38. [PubMed: 23100568]
5. Brunham RC, Rappuoli R. Chlamydia trachomatis control requires a vaccine. *Vaccine*. 2013; 31:1892–1897. [PubMed: 23375977]
6. Hafner LM, Wilson DP, Timms P. Development status and future prospects for a vaccine against Chlamydia trachomatis infection. *Vaccine*. 2014; 32:1563–1571. [PubMed: 23973245]
7. Roan NR, Starnbach MN. Immune-mediated control of Chlamydia infection. *Cell Microbiol*. 2008; 10:9–19. [PubMed: 17979983]
8. Rank RG, Whittum-Hudson JA. Protective immunity to chlamydial genital infection: evidence from animal studies. *J Infect Dis*. 2010; 201(Suppl 2):S168–S177. [PubMed: 20470052]
9. Cooper AM. Cell-mediated immune responses in tuberculosis. *Annu Rev Immunol*. 2009; 27:393–422. [PubMed: 19302046]
10. McSorley SJ. Immunity to intestinal pathogens: lessons learned from Salmonella. *Immunol Rev*. 2014; 260:168–182. [PubMed: 24942689]
11. Good MF, Xu H, Wykes M, Engwerda CR. Development and regulation of cell-mediated immune responses to the blood stages of malaria: implications for vaccine research. *Annu Rev Immunol*. 2005; 23:69–99. [PubMed: 15771566]
12. Morrison RP, Caldwell HD. Immunity to murine chlamydial genital infection. *Infect Immun*. 2002; 70:2741–2751. [PubMed: 12010958]
13. Casadevall A, Pirofski LA. A new synthesis for antibody-mediated immunity. *Nat Immunol*. 2012; 13:21–28. [PubMed: 22179281]
14. Rank RG, White HJ, Barron AL. Humoral immunity in the resolution of genital infection in female guinea pigs infected with the agent of guinea pig inclusion conjunctivitis. *Infect Immun*. 1979; 26:573–579. [PubMed: 546788]
15. Rank RG, Barron AL. Humoral immune response in acquired immunity to chlamydial genital infection of female guinea pigs. *Infect Immun*. 1983; 39:463–465. [PubMed: 6822430]
16. Rank RG, Batteiger BE. Protective role of serum antibody in immunity to chlamydial genital infection. *Infect Immun*. 1989; 57:299–301. [PubMed: 2909492]
17. Ramsey KH, Soderberg LS, Rank RG. Resolution of chlamydial genital infection in B-cell-deficient mice and immunity to reinfection. *Infect Immun*. 1988; 56:1320–1325. [PubMed: 3258586]
18. Batteiger BE, Rank RG. Analysis of the humoral immune response to chlamydial genital infection in guinea pigs. *Infect Immun*. 1987; 55:1767–1773. [PubMed: 3610314]
19. Ramsey KH, Newhall WJ, Rank RG. Humoral immune response to chlamydial genital infection of mice with the agent of mouse pneumonitis. *Infect Immun*. 1989; 57:2441–2446. [PubMed: 2744854]
20. 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. [PubMed: 9169723]

21. Johansson M, Ward M, Lycke N. B-cell-deficient mice develop complete immune protection against genital tract infection with *Chlamydia trachomatis*. *Immunology*. 1997; 92:422–428. [PubMed: 9497482]
22. Rank RG, Soderberg LS, Barron AL. Chronic chlamydial genital infection in congenitally athymic nude mice. *Infect Immun*. 1985; 48:847–849. [PubMed: 3997251]
23. Perry LL, Feilzer K, Caldwell HD. Immunity to *Chlamydia trachomatis* is mediated by T helper 1 cells through IFN- γ -dependent and -independent pathways. *J Immunol*. 1997; 158:3344–3352. [PubMed: 9120292]
24. 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. [PubMed: 7591120]
25. 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. [PubMed: 11083822]
26. Morrison SG, Morrison RP. Resolution of secondary *Chlamydia trachomatis* genital tract infection in immune mice with depletion of both CD4⁺ and CD8⁺ T cells. *Infect Immun*. 2001; 69:2643–2649. [PubMed: 11254630]
27. Morrison SG, Morrison RP. A predominant role for antibody in acquired immunity to chlamydial genital tract reinfection. *J Immunol*. 2005; 175:7536–7542. [PubMed: 16301662]
28. Leon B, Ballesteros-Tato A, Misra RS, Wojciechowski W, Lund FE. Unraveling effector functions of B cells during infection: the hidden world beyond antibody production. *Infect Disord Drug Targets*. 2012; 12:213–221. [PubMed: 22394173]
29. Punnonen R, Terho P, Kleimi PJ. Chlamydial pelvic inflammatory disease with ascites. *Fertil Steril*. 1982; 37:270–272. [PubMed: 7060774]
30. Marbet UA, Stalder GA, Vogtlin J, Loosli J, Frei A, Althaus B, et al. Diffuse peritonitis and chronic ascites due to infection with *Chlamydia trachomatis* in patients without liver disease: new presentation of the Fitz-Hugh-Curtis syndrome. *Br Med J (Clin Res Ed)*. 1986; 293:5–6.
31. Priego M, Merino E, Boix V, Portilla J. Pelvic inflammatory disease caused by *Chlamydia trachomatis* as a cause of ascites. *Enferm Infecc Microbiol Clin*. 2000; 18:202–203. [PubMed: 10932408]
32. Sran SD, Mautone AS, Kolomeyer AM, Cracchiolo BM, Heller DS, Pletcher BA. Diffuse peritoneal chlamydial infection presenting as possible ovarian peritoneal carcinomatosis in an adolescent female. *J Adolesc Health*. 2012; 50:531–533. [PubMed: 22525121]
33. Li LX, McSorley SJ. B Cells Enhance Antigen-Specific CD4 T Cell Priming and Prevent Bacteria Dissemination following *Chlamydia muridarum* Genital Tract Infection. *PLoS Pathog*. 2013; 9:e1003707. [PubMed: 24204262]
34. Peeling R, Maclean IW, Brunham RC. In vitro neutralization of *Chlamydia trachomatis* with monoclonal antibody to an epitope on the major outer membrane protein. *Infect Immun*. 1984; 46:484–488. [PubMed: 6209221]
35. Megran DW, Stiver HG, Peeling R, Maclean IW, Brunham RC. Complement enhancement of neutralizing antibody to the structural proteins of *Chlamydia trachomatis*. *J Infect Dis*. 1988; 158:661–663. [PubMed: 3411155]
36. Caldwell HD, Perry LJ. Neutralization of *Chlamydia trachomatis* infectivity with antibodies to the major outer membrane protein. *Infect Immun*. 1982; 38:745–754. [PubMed: 7141712]
37. Pal S, Theodor I, Peterson EM, de la Maza LM. Monoclonal immunoglobulin A antibody to the major outer membrane protein of the *Chlamydia trachomatis* mouse pneumonitis biovar protects mice against a chlamydial genital challenge. *Vaccine*. 1997; 15:575–582. [PubMed: 9160528]
38. Peterson EM, de la Maza LM, Brade L, Brade H. Characterization of a neutralizing monoclonal antibody directed at the lipopolysaccharide of *Chlamydia pneumoniae*. *Infect Immun*. 1998; 66:3848–3855. [PubMed: 9673271]
39. Peterson EM, Cheng X, Markoff BA, Fielder TJ, de la Maza LM. Functional and structural mapping of *Chlamydia trachomatis* species-specific major outer membrane protein epitopes by use of neutralizing monoclonal antibodies. *Infect Immun*. 1991; 59:4147–4153. [PubMed: 1718870]

40. Cotter TW, Meng Q, Shen ZL, Zhang YX, Su H, Caldwell HD. Protective efficacy of major outer membrane protein-specific immunoglobulin A (IgA) and IgG monoclonal antibodies in a murine model of *Chlamydia trachomatis* genital tract infection. *Infect Immun*. 1995; 63:4704–4714. [PubMed: 7591126]
41. Morrison RP, Belland RJ, Lyng K, Caldwell HD. Chlamydial disease pathogenesis. The 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J Exp Med*. 1989; 170:1271–1283. [PubMed: 2571668]
42. Brunham RC, Maclean IW, Binns B, Peeling RW. Chlamydia trachomatis: its role in tubal infertility. *J Infect Dis*. 1985; 152:1275–1282. [PubMed: 3840827]
43. Crane DD, Carlson JH, Fischer ER, Bavoi P, Hsia RC, Tan C, et al. Chlamydia trachomatis polymorphic membrane protein D is a species-common pan-neutralizing antigen. *Proc Natl Acad Sci U S A*. 2006; 103:1894–1899. [PubMed: 16446444]
44. Shaw EI, Dooley CA, Fischer ER, Scidmore MA, Fields KA, Hackstadt T. Three temporal classes of gene expression during the *Chlamydia trachomatis* developmental cycle. *Mol Microbiol*. 2000; 37:913–925. [PubMed: 10972811]
45. Bode J, Dutow P, Sommer K, Janik K, Glage S, Tümmler B, et al. A New Role of the Complement System: C3 Provides Protection in a Mouse Model of Lung Infection with Intracellular *Chlamydia psittaci*. *PLoS One*. 2012; 7:e50327. [PubMed: 23189195]
46. Dutow P, Fehlhaber B, Bode J, Laudeley R, Rheinheimer C, Glage S, et al. The complement C3a receptor is critical in defense against *Chlamydia psittaci* in mouse lung infection and required for antibody and optimal T cell response. *J Infect Dis*. 2014; 209:1269–1278. [PubMed: 24273177]
47. Yang Z, Conrad T, Zhou Z, Chen J, Dutow P, Klos A, et al. Complement factor C5 but not C3 contributes significantly to hydrosalpinx development in mice infected with *Chlamydia muridarum*. *Infect Immun*. 2014; 82:3154–3163. [PubMed: 24842924]
48. Moore T, Ananaba GA, Bolier J, Bowers S, Belay T, Eko FO, et al. Fc receptor regulation of protective immunity against *Chlamydia trachomatis*. *Immunology*. 2002; 105:213–221. [PubMed: 11872097]
49. Moore T, Ekworomadu CO, Eko FO, MacMillan L, Ramey K, Ananaba GA, et al. Fc receptor-mediated antibody regulation of T cell immunity against intracellular pathogens. *J Infect Dis*. 2003; 188:617–624. [PubMed: 12898452]
50. Haase AT. Targeting early infection to prevent HIV-1 mucosal transmission. *Nature*. 2010; 464:217–223. [PubMed: 20220840]
51. 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. [PubMed: 9686609]
52. Moseman EA, Iannacone M, Bosurgi L, Tonti E, Chevrier N, Tumanov A, et al. B cell maintenance of subcapsular sinus macrophages protects against a fatal viral infection independent of adaptive immunity. *Immunity*. 2012; 36:415–426. [PubMed: 22386268]
53. Taylor JJ, Pape KA, Jenkins MK. A germinal center-independent pathway generates unswitched memory B cells early in the primary response. *J Exp Med*. 2012; 209:597–606. [PubMed: 22370719]
54. Teitelbaum R, Glatman-Freedman A, Chen B, Robbins JB, Unanue E, Casadevall A, et al. A mAb recognizing a surface antigen of *Mycobacterium tuberculosis* enhances host survival. *Proc Natl Acad Sci U S A*. 1998; 95:15688–15693. [PubMed: 9861031]
55. Pethe K, Alonso S, Biet F, Delogu G, Brennan MJ, Locht C, et al. The heparin-binding haemagglutinin of *M. tuberculosis* is required for extrapulmonary dissemination. *Nature*. 2001; 412:190–194. [PubMed: 11449276]
56. Williams A, Reljic R, Naylor I, Clark SO, Falero-Diaz G, Singh M, et al. Passive protection with immunoglobulin A antibodies against tuberculous early infection of the lungs. *Immunology*. 2004; 111:328–333. [PubMed: 15009434]
57. Maglione PJ, Xu J, Chan J. B cells moderate inflammatory progression and enhance bacterial containment upon pulmonary challenge with *Mycobacterium tuberculosis*. *J Immunol*. 2007; 178:7222–7234. [PubMed: 17513771]

58. Kozakiewicz L, Chen Y, Xu J, Wang Y, Dunussi-Joannopoulos K, Ou Q, et al. B Cells Regulate Neutrophilia during Mycobacterium tuberculosis Infection and BCG Vaccination by Modulating the Interleukin-17 Response. *PLoS Pathog.* 2013; 9:e1003472. [PubMed: 23853593]
59. Mastroeni P, Simmons C, Fowler R, Hormaeche CE, Dougan G. Igh-6^{-/-} (B-cell-deficient) mice fail to mount solid acquired resistance to oral challenge with virulent Salmonella enterica serovar typhimurium and show impaired Th1 T-cell responses to Salmonella antigens. *Infect Immun.* 2000; 68:46–53. [PubMed: 10603367]
60. McSorley SJ, Jenkins MK. Antibody is required for protection against virulent but not attenuated Salmonella enterica serovar typhimurium. *Infect Immun.* 2000; 68:3344–3348. [PubMed: 10816483]
61. Nanton MR, Way SS, Shlomchik MJ, McSorley SJ. Cutting Edge: B Cells Are Essential for Protective Immunity against Salmonella Independent of Antibody Secretion. *J Immunol.* 2012; 189:5503–5507. [PubMed: 23150714]
62. Cunningham AF, Gaspal F, Serre K, Mohr E, Henderson IR, Scott-Tucker A, et al. Salmonella induces a switched antibody response without germinal centers that impedes the extracellular spread of infection. *J Immunol.* 2007; 178:6200–6207. [PubMed: 17475847]
63. Barr TA, Brown S, Mastroeni P, Gray D. TLR and B cell receptor signals to B cells differentially program primary and memory Th1 responses to Salmonella enterica. *J Immunol.* 2010; 185:2783–2789. [PubMed: 20675594]
64. Talbot S, Totemeyer S, Yamamoto M, Akira S, Hughes K, Gray D, et al. Toll-like receptor 4 signalling through MyD88 is essential to control Salmonella enterica serovar typhimurium infection, but not for the initiation of bacterial clearance. *Immunology.* 2009; 128:472–483. [PubMed: 19930040]
65. Mastroeni P, Villarreal-Ramos B, Hormaeche CE. Adoptive transfer of immunity to oral challenge with virulent salmonellae in innately susceptible BALB/c mice requires both immune serum and T cells. *Infect Immun.* 1993; 61:3981–3984. [PubMed: 8359920]
66. Humphres RC, Hinrichs DJ. Role of antibody in Coxiella burnetii infection. *Infect Immun.* 1981; 31:641–645. [PubMed: 7216465]
67. Shannon JG, Cockrell DC, Takahashi K, Stahl GL, Heinzen RA. Antibody-mediated immunity to the obligate intracellular bacterial pathogen Coxiella burnetii is Fc receptor- and complement-independent. *BMC immunology.* 2009; 10:26. [PubMed: 19426498]
68. Zhang G, Russell-Lodrigue KE, Andoh M, Zhang Y, Hendrix LR, Samuel JE. Mechanisms of vaccine-induced protective immunity against Coxiella burnetii infection in BALB/c mice. *J Immunol.* 2007; 179:8372–8380. [PubMed: 18056383]
69. Li JS, Yager E, Reilly M, Freeman C, Reddy GR, Reilly AA, et al. Outer membrane protein-specific monoclonal antibodies protect SCID mice from fatal infection by the obligate intracellular bacterial pathogen Ehrlichia chaffeensis. *J Immunol.* 2001; 166:1855–1862. [PubMed: 11160232]
70. Li JS, Winslow GM. Survival, replication, and antibody susceptibility of Ehrlichia chaffeensis outside of host cells. *Infect Immun.* 2003; 71:4229–4237. [PubMed: 12874298]
71. McEwan WA, Tam JC, Watkinson RE, Bidgood SR, Mallery DL, James LC. Intracellular antibody-bound pathogens stimulate immune signaling via the Fc receptor TRIM21. *Nat Immunol.* 2013; 14:327–336. [PubMed: 23455675]
72. Rakebrandt N, Lentès S, Neumann H, James LC, Neumann-Staubitz P. Antibody- and TRIM21-dependent intracellular restriction of Salmonella enterica. *Pathog Dis.* 2014; 72:131–137. [PubMed: 24920099]
73. Farris CM, Morrison RP. Vaccination against Chlamydia genital infection utilizing the murine C. muridarum model. *Infect Immun.* 2011; 79:986–996. [PubMed: 21078844]
74. Yu H, Karunakaran KP, Jiang X, Shen C, Andersen P, Brunham RC. Chlamydia muridarum T cell antigens and adjuvants that induce protective immunity in mice. *Infect Immun.* 2012; 80:1510–1518. [PubMed: 22290151]
75. Yu H, Karunakaran KP, Jiang X, Brunham RC. Evaluation of a multisubunit recombinant polymorphic membrane protein and major outer membrane protein T cell vaccine against Chlamydia muridarum genital infection in three strains of mice. *Vaccine.* 2014; 32:4672–4680. [PubMed: 24992718]

76. Sun G, Pal S, Weiland J, Peterson EM, de la Maza LM. Protection against an intranasal challenge by vaccines formulated with native and recombinant preparations of the *Chlamydia trachomatis* major outer membrane protein. *Vaccine*. 2009; 27:5020–5025. [PubMed: 19446590]
77. Kari L, Whitmire WM, Crane DD, Reveneau N, Carlson JH, Goheen MM, et al. *Chlamydia trachomatis* native major outer membrane protein induces partial protection in nonhuman primates: implication for a trachoma transmission-blocking vaccine. *J Immunol*. 2009; 182:8063–8070. [PubMed: 19494332]
78. Pal S, Theodor I, Peterson EM, de la Maza LM. Immunization with an acellular vaccine consisting of the outer membrane complex of *Chlamydia trachomatis* induces protection against a genital challenge. *Infect Immun*. 1997; 65:3361–3369. [PubMed: 9234798]
79. Teng A, Cruz-Fisher MI, Cheng C, Pal S, Sun G, Ralli-Jain P, et al. Proteomic identification of immunodominant chlamydial antigens in a mouse model. *J proteomics*. 2012; 77:176–186. [PubMed: 22959960]
80. Wang J, Zhang Y, Lu C, Lei L, Yu P, Zhong G. A genome-wide profiling of the humoral immune response to *Chlamydia trachomatis* infection reveals vaccine candidate antigens expressed in humans. *J Immunol*. 2010; 185:1670–1680. [PubMed: 20581152]
81. Patton DL, Teng A, Randall A, Liang X, Felgner PL, de la Maza LM. Whole genome identification of *C. trachomatis* immunodominant antigens after genital tract infections and effect of antibiotic treatment of pigtailed macaques. *J proteomics*. 2014; 108:99–109. [PubMed: 24862987]

Figure 1.

Potential role of B cells in the draining iliac lymph nodes during *Chlamydia* genital tract infection. (A) B cells might act directly as antigen presenting cells to T cells, (B) help maintain the lymphoid architecture perhaps to allow SCS macrophages to function in anti-bacterial immunity upon active *Chlamydia* infection, (C) directly contribute to early defense by producing inflammatory cytokines in response to bacterial ligands or, (D) rapidly produce antibody in the draining lymph node that hinders pathogen spread.