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Hypothesis: *Chlamydia trachomatis* infection of the female genital tract is controlled by T_H2 immunity

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Abstract

Chlamydia trachomatis is an obligate intracellular bacterium sexually transmitted to more than 90 million individuals each year. As this level of infectivity implies, *C. trachomatis* is a successful human parasite; a success facilitated by its ability to asymptotically infect women. Host defense against *C. trachomatis* in the female genital tract is not well defined, but current dogma suggests infection is controlled largely by T_H1 immunity. Conversely, it is well established that T_H2 immunity controls allergens, helminths, and other extracellular pathogens that cause repetitive or persistent T cell stimulation but do not induce the exuberant inflammation that drives T_H1 and T_H17 immunity. As *C. trachomatis* persists in female genital tract epithelial cells but does not elicit overt tissue inflammation, we now posit that defense is maintained by T_H2 immune responses that control bacterial growth but minimize immunopathological damage to vital reproductive tract anatomy. Evaluation of this hypothesis may uncover novel mechanisms by which T_H2 immunity can control growth of *C. trachomatis* and other intracellular pathogens, while confirmation that T_H2 immunity was selected by evolution to control *C. trachomatis* infection in the female genital tract will transform current research, now focused on developing vaccines that elicit strong, and therefore potentially tissue destructive, *Chlamydia*-specific T_H1 immunity.

INTRODUCTION

Host defense

Protection against microbial pathogens is orchestrated by a coordinated system in which innate immune responses supply the first lines of defense and drive the formation of more specialized adaptive immune responses. Depending on the infection, dendritic cells (DC) and other innate immune cells express distinct sets of costimulatory molecules and cytokines that trigger naïve CD4⁺ T cells to form various types of effector cells, three of which are termed T_H1, T_H2, and T_H17 [1]. Specifically, many intracellular bacteria and viruses induce DC and natural killer (NK) cells to produce interleukin (IL)-12 and interferon (IFN)- γ , which drives T_H1 differentiation; helminths and other extracellular pathogens cause innate immune cells to secrete IL-4, which promotes T_H2 differentiation; while other bacteria and

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fungi stimulate DC to produce IL-1, IL-6, and transforming growth factor- β , which induces T_H17 differentiation [2-5]. Such specialized adaptive immune responses allow animal hosts to optimize their defenses against a diverse array of naturally encountered pathogens.

Naïve $CD4^+$ T cells stimulated to form T_H1 cells then secrete high levels of IFN- γ , activating macrophages (called classical macrophage activation) to phagocytose and eliminate the microbes that triggered T_H1 development [6]. T_H1 $CD4^+$ T cells also secrete tumor necrosis factor and proinflammatory chemokines that recruit additional leukocytes to active infection sites [7]. However, when T_H1 immunity cannot adequately control infection, host defense may be polarized toward T_H2 immune responses that curb T_H1 -mediated inflammation and inhibit collateral tissue damage [8]. For example, helminths and other extracellular pathogens refractory to phagocytic killing stimulate T_H2 $CD4^+$ T cells to secrete IL-4, IL-11, and IL-13, responses that: inhibit T_H1 and T_H17 cell development, decrease inflammatory cell recruitment, and activate macrophages to promote tissue remodeling and wound healing (called alternative macrophage activation) [9, 10].

Endocervical *Chlamydia trachomatis* infection

Chlamydia trachomatis is an obligate intracellular bacterium, and exclusively a pathogen of humans. With at least 90 million new cases globally each year, this microorganism is the most common sexually transmitted bacterial infection [11]. The highest prevalences of genital tract *Chlamydia* infection are found among adolescents and young adults, and migration of the organism from the lower to upper female genital tract can cause Fallopian tube damage, and increase the risk for ectopic pregnancy and tubal factor infertility [12]. Persistent infection is another risk factor for phenotypic expression of chlamydial disease [13], and is facilitated by the ability of *C. trachomatis* to asymptomatically infect women [14]. In fact, without administration of an appropriate antimicrobial, this organism can persist subclinically in genital tract epithelium for years after initial infection [15]. Interestingly, chlamydial infection control programs that augment detection and treatment of asymptomatically individuals concomitantly increase populational susceptibility to infection, suggesting that an earlier eradication of the organism interrupts development of slow-to-form *Chlamydia*-specific protective immunity [16-18]. Moreover, even though *C. trachomatis* is a Gram-negative bacterium, its cell wall contains lipopolysaccharide (LPS) that is at least 100-fold less potent than LPS contained in *Neisseria gonorrhoeae*, a Gram-negative bacterium that elicits more robust genital tract inflammation [19]. Collectively, these clinical, epidemiological, and laboratory findings indicate that *C. trachomatis* is a weakly antigenic parasite that elicits mild inflammatory responses and asymptomatically persists in the female genital tract; thus optimizing its chances for transmission and its survival fitness.

HYPOTHESIS

As T_H2 differentiation is promoted by weakly antigenic pathogens and pathogens refractory to eradication by T_H1 and T_H17 immunity, we posit that defense against *C. trachomatis* in the female genital tract is ultimately regulated by T_H2 immune responses that control chlamydial replication and suppress immunopathological damage to vital reproductive tract structures (Figure).

TESTING THE HYPOTHESIS

To begin exploration of this hypothesis, we completed immunohistochemical analyses, with antibodies specific for macrophages, NK cells, T cells, B cells, and plasma cells, to characterize the leukocyte subpopulations in paraffin-embedded endometrial tissue from 37 uninfected women and 27 women with *C. trachomatis* genital tract infection. Among

Chlamydia-infected study participants, 17 were diagnosed with endocervical infection alone, and 10 were diagnosed with both endocervical and endometrial infection [20]. Compared to uninfected women, we found that endocervical *C. trachomatis* infection was associated with significant increases in endometrial T cells, B cells, and plasma cells. As the numbers of endometrial stromal cells positive for CD3 (a pan T cell marker), but not CD8, were increased among these women, our results also implied that CD4⁺ cells were the T cell subset specifically increased by endocervical *C. trachomatis* infection. Ascension of *C. trachomatis* into the upper genital tract elicited even more substantial increase in the numbers of endometrial CD4⁺ T cells, B cells, and plasma cells compared to when infection was confined to the lower genital tract, although infection in these women was also asymptomatic. While these investigations confirm that cell mediated immunity, including increases in CD4⁺ T cell numbers, are elicited by *C. trachomatis* infection of the female genital tract, further work is needed to determine the CD4⁺ effector cell subsets specifically selected by evolution to combat *Chlamydia* infection.

Future studies exploring our hypothesis should define which of the transcription factors regulating T cell differentiation are preferentially expressed among CD4⁺ cells infiltrating the genital tract of women with *C. trachomatis* infection. T_H1 cell differentiation, for example, is associated with expression of T-bet, a transcription factor that is considered the master regulator of the T_H1 response [21]. Similarly, T_H2 and T_H17 immunity is driven by host responses that increase expression of the transcription factors GATA-3 and RORγt, respectively [22]. We hypothesize that acquisition of *C. trachomatis* is associated, at first, with T_H1 differentiation, and increased T-bet expression by genital tract CD4⁺ T cells. However, as T_H1 immunity cannot prevent *C. trachomatis* from establishing a persistent genital tract infection, host defense is increasing polarized toward T_H2 responses that dampen tissue inflammation and inhibit collateral damage to vital reproductive tract anatomy. Thus we also hypothesize that CD4⁺ T cells in the genital tract of women with chronic *C. trachomatis* infection will preferentially express GATA-3, the master regulator of T_H2 immunity. If our hypotheses are correct, it will then be important to explore if *Chlamydia*-infected epithelial cells, or perhaps other innate immune cells, are responsible for inducing this T_H2 differentiation.

Accordingly, the effector function of *Chlamydia*-specific CD4⁺ memory T cells isolated from the blood and genital tract of women with prior or existing genital tract infection should also be examined. As we predict that initial acquisition of *C. trachomatis* elicits T_H1 immunity, a fraction of these CD4⁺ memory T cells from these women are expected to produce IFN-γ in response to *ex vivo* stimulation with chlamydial antigen. However, as we hypothesize that host defense against *C. trachomatis* becomes increasingly polarized toward T_H2 immunity, a significant number of *Chlamydia*-specific CD4⁺ memory cells are expected to exhibit a T_H2 phenotype and produce IL-4, IL-5, and IL-13 in response to similar stimulation. We further predict that differentiation of T_H2 immune responses in women with persistent *C. trachomatis* infection will stimulate macrophages to promote tissue remodeling and repair (as opposed to IFN-γ activated macrophages, which show increased microbicidal function) [23-25].

If the studies outlined above determine that host defense in the genital tract of *Chlamydia*-infected women becomes polarized toward T_H2 immunity, other work would need to define if T_H2 immunity also controls *C. trachomatis* replication in genital tract epithelial cells. Previous studies showed the IFN-γ controls growth of *Chlamydia* species *in vitro* by inducing the production of indoleamine-2, 3-dioxygenase, an enzyme that degrades tryptophan, and hinders *Chlamydia* spp. growth by tryptophan starvation [26]. However, there is little evidence to support that *Chlamydia*-specific T_H1 immunity is a biologically relevant response in the genital tract of women, and that chlamydial growth is controlled *in*

vivo by this mechanism. Among the few studies to examine this question, commercial sex workers in Kenya whose peripheral blood mononuclear cells produced IFN- γ in response to *ex vivo* stimulation with chlamydial antigen had increased resistance to *C. trachomatis* genital tract re-infection, but this study was unable to assess if these protective T_H1 responses were also associated with increased genital tract inflammation and collateral tissue damage [27]. However, there is equally little data supporting greater relevance of another CD4⁺ T cell subset. Thus, future studies will also need to examine the possibility that T_H2 immunity was selected by evolution to defend against *C. trachomatis* infection in women as a means to prevent prolonged or excessive inflammation from damaging reproductive tract tissue. In conjunction with such studies, however, is the need to explore the possibility that T_H2 immune responses can regulate *C. trachomatis* replication. Although we hypothesize that T_H2 immunity will not curb *Chlamydia* growth as effectively as T_H1-mediated tryptophan starvation, such work has the potential to uncover novel, biologically relevant mechanisms by which T_H2 immunity inhibits growth of *Chlamydia* and other intracellular bacteria. Such possibilities are, of course, now speculative, but they are consistent with observations that indicate *C. trachomatis* genital tract infection of women is asymptomatic, maintained long-term at low levels of bacterial burden, and only slowly cleared in the absence of antimicrobial administration.

HYPOTHESIS IMPLICATIONS FOR CHLAMYDIAL PATHOGENESIS

If correct, our hypothesis would transform prophylactic *C. trachomatis* vaccine development, as much of its current focus is based on the results from murine models of infection that suggest strong *Chlamydia*-specific T_H1 immunity will be an essential element of an effective vaccine. However, murine infection rather imprecisely models the pathogenesis of human *C. trachomatis* endocervical infection. In the first place, most of these mice were intravaginally infected with *C. muridarum*, a chlamydia species that is not a natural pathogen of the murine reproductive tract [28]. Perhaps because *C. muridarum* did not evolve as a murine genital tract pathogen, intravaginal infection stimulates strong innate immune responses and the differentiation of T_H1 (primarily) and T_H17 immunity [29]. This vigorous host response typically clears *C. muridarum* within 1 month of infection, unlike *C. trachomatis*, which is more likely to cause chronic infection in women [30]. In the process of eradicating experimental intravaginal infection, T_H1 immunity against *C. muridarum* also causes significant damage to the upper genital tract of mice, including frequent development of hydrosalpinx (a dilated, fluid-filled Fallopian tube) [31]. This is in stark contrast to endocervical infection of women, in which *C. trachomatis* is only infrequently responsible for upper genital tract damage [32]. Therefore, use of the results from murine models to draw conclusions about the response of women to endocervical *C. trachomatis* infection has likely overestimated the biological relevance of T_H1 immunity.

Although murine infection inadequately models endocervical *C. trachomatis* infection of women, it has been useful to re-enforce the concepts that animal defense mechanisms co-evolved with a defined set of pathogens and hosts respond to these pathogens in ways that are optimal for host survival fitness. The T_H1 response to *C. muridarum* rapidly clears infection, but is inappropriate, as it was not selected by evolution to prevent Fallopian tube damage [33]. The response to *C. trachomatis* in the genital tract of women, on the other hand, was selected to prevent uncontrolled bacterial replication from damaging epithelium as well as exuberant inflammation from causing immunopathological destruction of reproductive tract tissue. Because T_H2 immunity inhibits the development of more tissue-destructive T_H1 and T_H17 immunity, it may have been the response selected by evolution to combat *C. trachomatis* and other persistent genital tract infections. Although the function of T_H2 immunity in defense against allergens, helminths, and other extracellular pathogens is established, we now hypothesize that T_H2 immune responses also dampen the intensity of

inflammation elicited by chronic *C. trachomatis* infection while also controlling the growth of this obligate intracellular bacterium in epithelial tissue of the female genital tract.

HYPOTHESIS IMPLICATIONS FOR VACCINE DEVELOPMENT

C. trachomatis is a ubiquitous sexually transmitted pathogen with a predilection to establish persistent infection, and host defenses in the female genital tract evolved in the constant presence of this bacterium. To optimize the opportunities for survival and transmission, *C. trachomatis* also evolved to elicit responses that suppress, but do not disarm, host defense. It is quite possible, therefore, that both the character and strength of the immune responses elicited against other sexually transmitted pathogens have been adjusted by evolution to counter the diminution of genital tract inflammatory responses that is sequelae to persistent *Chlamydia* infection. If our hypothesis is correct, and *C. trachomatis* stimulates polarization toward T_H2 immunity and alternative macrophage activation, then a prophylactic chlamydial vaccine administered to women prior to sexual debut has the potential to heighten reactivity to other sexually transmitted microorganisms, and increase the possibility for immunopathological genital tract damage. Less speculative, development of vaccines that induce strong *Chlamydia*-specific T_H1 memory would similarly increase the probability for collateral tissue damage upon repetitive exposure to *C. trachomatis* in the female genital tract. Therefore, as ectopic pregnancy and tubal factor infertility are less frequent consequences of *C. trachomatis* infection in areas of the world that maintain chlamydial infection control programs, development of vaccines that suppress phenotypic expression of disease, versus vaccines that stimulate *Chlamydia*-specific T_H1 memory, may warrant greater emphasis. Though these implications of our hypothesis are both plausible and thought provoking, the more basic and immediate objective should be the elucidation of the specific effector CD4⁺ T cell subsets induced by genital tract *C. trachomatis* infection of women, as this is a prerequisite for defining the optimal approach to *Chlamydia* vaccine development.

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1. . Cartoon depiction of host defense against *Chlamydia trachomatis* infection of the female genital tract

Although *C. trachomatis* acquisition may initially induce T_H1 immune responses, we hypothesize that persistent infection elicits responses that suppress T_H1 immunity and polarize host defense toward T_H2 immunity. In response to persistent infection, we further hypothesize that T_H2 CD4⁺ T cells secrete cytokines, which stimulate *Chlamydia*-specific antibody production, inhibit chlamydial growth, and activate macrophages that dampen inflammation and promote tissue repair.