

ASPECTS OF REPRODUCTIVE BIOLOGY THAT INFLUENCE THE DISTRIBUTION AND SPREAD OF *CHLAMYDIA TRACHOMATIS* WITHIN THE FEMALE GENITAL TRACT: A NEW PARADIGM

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SUMMARY

Critical to evaluating Chlamydia trachomatis vaccine candidates is the availability of appropriate animal models. At a minimum, models must mimic the essential features of

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transmission and disease progression that contribute to the severe outcomes associated with upper genital tract infection. Existing models, whether mouse, pig or nonhuman primate, are based on the generally accepted premise that upper genital tract infection, when it occurs, is an event subsequent to cervical infection. However, what this simple paradigm overlooks are many features of reproduc-

tive biology that could influence both the initial distribution and subsequent spread of *C. trachomatis* within the female genital tract, as well as the immune responses made at these site(s) of infection. A review of the literature strongly suggests that the menstrual cycle and coitus-related phenomena are likely to have a profound effect on the course and outcome of female genital tract infection with *C. trachomatis*. Although the new paradigm that emerges raises concerns about the adequacy of existing animal models, it also suggests ways to modify these models to better mimic the complexities of human infection and therefore serve as appropriate models in which to test the safety and efficacy of vaccine candidates against *C. trachomatis* infection in women.

INTRODUCTION

Since its conception a decade ago, the integrated approach to the study of *Chlamydia trachomatis* infection of the female genital tract (1) has been successful in its initial attempts: i) to identify and test candidate genes that are associated with susceptibility to and outcome of infection (2, 3); and ii) to promote the use of human urogenital serovars in animal and in vitro models in order to improve the translational value of these efforts (4, 5). However, with a number of *C. trachomatis* vaccine candidates ready for preclinical safety and efficacy testing, the ultimate challenge for the integrated approach will be to suggest and, if necessary, develop the appropriate animal models for use in this effort. At a minimum, these models must mimic the essential features of transmission and disease progression that contribute to the severe outcomes associated with upper genital tract infection with this agent, in order to eliminate the risk of inadvertently promoting these sequelae following vaccination.

Currently used animal models, whether mouse, pig or nonhuman primate, have been based on the generally accepted premise that upper genital tract infection, when it occurs, is a linear ascending process that originates with an aggressive and/or persistent cervical infection (6). As a result, the models are static with respect to the issue of transmission of *C. trachomatis* between partners during intercourse, with the direct installation of elementary bodies (EBs) into the vagina being considered an adequate representation of this event (7). However, what this simple paradigm overlooks are many features of reproductive anatomy, physiology and immunology that could influence the spread of *C. trachomatis* within the female genital tract. In fact, a

review of the literature strongly suggests that many natural processes associated with human reproductive biology are likely to have a dynamic influence on the distribution and spread of *C. trachomatis* within the female genital tract, both coincidental to intercourse and during localized cervical and upper genital tract infection. In addition, aspects of these processes that are designed to promote conception, successful implantation and carriage of the fetus to term, almost certainly play a role in the immune responses made to infectious agents present in both semen and at infected sites within the female genital tract.

This paper will describe some of the likely effects that the evolutionarily connected but distinguishable features of the menstrual cycle and coitus-related phenomena have on the susceptibility, course and outcome of female genital tract infection (GTI) with *C. trachomatis*. In the descriptions of the phenomena that follow, the reader should keep in mind two scenarios relative to the distribution and spread of *C. trachomatis* within the female genital tract: i) the postcoital distribution of *C. trachomatis* contained in seminal plasma of an infected male partner; and ii) the sexual activity-assisted spread of *C. trachomatis* from an infected site(s) to other locations within the female genital tract. Although the new paradigm of pathogenesis that emerges from this more comprehensive and integrated understanding raises concerns about the adequacy of existing animal models, it suggests ways to modify these models to better mimic the complexities and dynamics of human infection, thus serving as appropriate models in which to test the safety and efficacy of vaccine candidates against *C. trachomatis* infection of the female genital tract.

SEXUAL AROUSAL

Upon physical stimulation and/or the anticipation of sexual gratification, the female genital tract responds in ways that evolved to assist in the reproduction of the species, and hence with promoting receptivity to the male and promoting the transport of sperm deposited in the posterior vagina to the egg while it is in transit through the oviduct (8). This is achieved by an orchestrated series of contractions and menstrual cycle-associated peristaltic activity throughout the female genital tract, beginning with those that occur prior to coitus and via tactile foreplay, visual stimuli and mental fantasy (9, 10). Although less specifically directional at the outset, these contractions create movement within the vaginal and uterine walls, which when continued to orgasm increase

in both frequency and intensity (11). These contractions, along with the menstrual cycle-associated peristalsis described in a following section, would likely cause detachment of loosely associated cells along cervical and uterine epithelium, mixing of the luminal contents, and the possible mingling of both at the cervical–uterine junction. Under normal circumstances these contractions would occur without consequence. However, in the case of a woman infected with *C. trachomatis*, or any sexually transmitted disease (STD) agent, such activities may assist the ascending spread of infection into the upper genital tract (uterus and fallopian tubes), or reinfection of the cervix with elementary bodies shed from a site of infection in the upper genital tract. There is nothing static about this possible mechanism of *C. trachomatis* spread within the female genital tract or of any of the phenomena described in the following sections.

COITUS

Even in the absence of the preparatory responses described above, which set in motion stages of menstrual cycle-specific uterine contractions, the inward thrusting motion of an erect penis creates stretching of the vagina, and compression of the cervix and lower uterus, that when released between thrusts creates a suction-pump like effect between the vagina and uterus (12). During this process the vaginal, cervical and uterine contents are mingled along with detached cells and mucus, with the chance of vaginal and cervical contents reaching the uterotubal junction and entering the oviduct during the tubal contractions induced by repeated vaginal distention and cervical buffeting (13). An orgasm by either partner would heighten the contractions and their consequences and provide semen an opportunity to enter the uterus during postejaculatory thrusting (11, 13). In the case where the male partner uses a condom, there is no risk except for the possibility of physical irritation or damage to the vaginal and cervical epithelium that could increase the risk of inflammation and infection. Although as above, a *C. trachomatis*-infected woman would be at an increased risk of exposing the entire upper genital tract to both infectious EB and/or antigen-containing cellular debris that could, in the former case, initiate infection at a new site and, in the latter, could activate immune responsive cells at previous sites of infection. However, in the case of unprotected heterosexual coitus where the male partner is infected and shedding EB, the likely outcome in an uninfected female partner is clear – possible exposure to and infection with *C. trachomatis* at one or more sites throughout the entire

genital tract. While in a previous or currently infected female partner, reinfection and reexposure of active or previous sites of infection is possible.

MENSTRUAL CYCLE

The multiplicity of hormonally directed physical, physiological and immunological changes that occur during the menstrual cycle to promote successful reproduction, are also likely participants in the dynamic distribution and spread of *C. trachomatis* within the genital tract, both initially upon exposure to infected semen as well as during the course of an existing infection. Independent of any sexual activity, each of the distinct anatomical segments of the genital tract undergoes stage of cycle-associated changes in the intensity and direction of peristaltic motion appropriate to the stage of cycle function it supports (14, 15). This is at no other time more dramatically exemplified than the dominant follicle biased and directional peristalsis that occurs in the uterus during the follicular and ovulatory phases of the cycle, which in frequency and intensity acts in concert with follicle maturation and ovum release to assist sperm transport into the ovum-containing oviduct (16, 17) and perhaps coincidentally to a biased risk of unilateral tubal infection. Equally impressive in its function and possible effects on limiting the distribution and spread of *C. trachomatis* is the directionally biased fundus to cervix peristalsis that with increasing frequency and intensity occurs during the luteal phase, which reaches a maximum at menses (18, 19). In all its characteristics, this phase of the cycle would likely provide the greatest obstacle to initial *C. trachomatis* infection of the upper genital tract or spread from sites of infection in the lower tract during sexual activity.

Finally and apart from the focus on contractions and peristalsis, it should be mentioned that during the menstrual cycle the ability of exposed epithelial surfaces at different locations throughout the female genital tract to support productive infection with *C. trachomatis* varies from very hospitable to completely inhospitable. In addition, accessibility of the upper genital tract to either *C. trachomatis*-containing semen or secretions cycles through periods from probable to unlikely. When taken together, the current understanding of the cyclic changes that occur within the female genital tract would suggest that the upper genital tract is most accessible and susceptible during the late follicular and ovulatory phases, while becoming less accessible and inhospitable as the luteal phase progresses. During the follicular and

ovulatory phases, a metabolically and mitotically active epithelium provides the conditions required for productive infection with *C. trachomatis*, and the risk of tubal involvement is at its greatest for the reasons described above. While as the luteal phase progresses, the uterine epithelium degenerates and the edema and blood that engorge the uterus as menses approaches make productive infection difficult to establish or maintain. Additionally, the less hydrated and more impermeable cervical mucus (20, 21), the blockage of the oviducts by endometrial edema (22), and the less intense sexual activity-stimulated contractions (11) and a counter current peristaltic activity (23) would significantly reduce the risk of tubal exposure during this phase of the cycle.

SEMINAL PLASMA

Independent of all of the preceding mechanisms that improve receptivity and fertilization efficiency, and which *C. trachomatis* may have co-evolved to co-opt, are the components of semen that independently promote relaxation of the cervix, and coincidental and coordinated contractions of the entire genital tract, particularly the cervix and uterus. Seminal plasma contains the highest concentrations of oxytocin and prostaglandins (PG) of any fluid secretion in men (24, 25). Oxytocin is a potent inducer of genital tract contractions (26, 27), while PGE₂ promotes cervical relaxation (28), and in conjunction with PGF₂ induces uterine contractions (29, 30). This has the obvious effect of enhancing seminal plasma uptake by and transport through the cervix and into the uterus and tubes (27, 29), likely increasing the risk of uterine and tubal infection both coincidental with coitus and through the spread from infection sites lower in the genital tract.

DISCUSSION

C. trachomatis is the most prevalent sexually transmitted bacterial infection worldwide. In order to control the infection, its complications and its transmission, it is crucial to develop new effective intervention strategies, principle among these being a safe and effective vaccine. However, it is important that the animal models used in preclinical assessments mimic the features of initial distribution and postinfection spread that take place in both previously uninfected as well as currently infected women. This is particularly critical for *C. trachomatis* GTI because of the suspected immunopathologic component associated with the most severe sequelae of infection.

The well-documented descriptions of events associated with the complex interplay between sexual activity and the menstrual cycle cited in this review support a new paradigm of upper genital tract exposure and infection that asserts that this site, no less than the cervix, can be a site of initial infection and not just a secondary site consequent to cervicitis. In addition, it provides a mechanism for the distribution throughout the genital tract of products of infection that derive from any site of infection or that are contained within infected semen deposited in the vagina during coitus. Given the strength of this research from fields outside of the usual purview of investigators in the field of STD research, it is our hope that acceptance of the many physical components of reproductive biology that likely affect *C. trachomatis* GTI, together with an appreciation of the possibly more significant impact of the immune modulation that occurs within the female genital tract during the menstrual cycle that is augmented by components of seminal plasma (manuscript in preparation), will encourage the development of animal models that better meet the more demanding needs of vaccine development. We are currently designing experiments to test a modified mouse model that will vaginally deposit *C. trachomatis* elementary body-containing seminal plasma stimulant (31) during intercourse simulating physical distention of the vagina and at known menstrual cycle equivalent phases of the estrus cycle. These same modifications could be made to both the pig and nonhuman primates models, making these species available for more translationally valuable vaccine testing.

Finally and in keeping with our overarching goal of integrating all available host, environmental and pathogen-related factors into a comprehensive and coherent understanding of female genital tract infection with *C. trachomatis*, it is interesting to speculate on how the new paradigm might help to identify critical events that contribute to upper genital infection and repeat exposure in those cases where infection is implicated causally in pelvic inflammatory disease (PID) and the more devastating sequelae of ectopic pregnancy and tubal factor infertility (Table I).

Although the question of how *C. trachomatis* might ascend in the human genital tract has not been specifically addressed in the cervicitis anchored paradigm, the inference that could be drawn from the literature on human disease progression and from the animal models based on this paradigm would suggest a static process of site to site progression "up" the genital tract without

Table 1. Risk factors associated with pelvic inflammatory disease (32-34).

Being a sexually active woman younger than 25 years of age
Having or being exposed to a sexually transmitted disease
Having multiple sexual partners or a partner with multiple partners
Frequent intercourse
Using nonbarrier contraceptives
Having an intrauterine device inserted
Douching regularly
Induced abortions
History of pelvic inflammatory disease

reference to how the physical, physiologic and immunologic dynamics of intercourse or the menstrual cycle affect this process. However, by taking these well-documented phenomena into account, STD agents including *C. trachomatis* are able to take advantage of numerous host-provided ways to become quickly distributed throughout the entire genital tract, coincidental with transmission in infected semen, as well as from a site of infection within the lower genital tract to other sites more distal. Of importance with respect to a condition like PID, where an immunopathologic component is implicated, these processes could also provide an infection-free mechanism of immune reactive cell exposure to antigens derived from semen and/or an active site of infection in the lower genital tract.

Among many hypothetical permutations, what this paradigm would predict is that a previously uninfected woman could experience a productive tubal infection in the absence of an existing cervicitis, while being at risk of tubal exposure to and infection with STD agents derived from active lower genital infection during each act of intercourse and during the follicular and ovulatory phases of every menstrual cycle. Once infected, local immune responses would be induced that would lead to either eradication or persistence and an immunologic memory that would respond to future encounters with the agent or its antigens in antigen-specific and stage of menstrual cycle determined ways. It would also suggest that in the case where the PID is caused by a response to a common bacterial and/or self-antigen, such as a member of the highly conserved heat shock protein family, that the opportunity for inducing a response increases proportionally to a woman's engaging in identified high risk behaviors and, once established, at sites within the host

where the common antigen is produced either naturally or pathologically.

CONCLUSIONS

Because of the serious outcomes associated with *C. trachomatis* infection in women, it is imperative as we advance chlamydial vaccine candidates for use in humans that we understand the nature of the events that contribute to tubal exposure to this agent and the nature of the immune responses made against it that contribute to the severe pathology associated with infection at this site. Thus, models based on the currently accepted paradigm of ascending spread secondary to cervicitis might be unproductive and possibly endangering, if the new paradigm being proposed contains elements that correctly capture the dynamic processes associated with the distribution and spread of this agent within the female genital tract. Therefore, attention must be given to the development of animal models of female genital tract infection with *C. trachomatis* that embody these elements.

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DISCLOSURE

The authors have nothing to disclose.

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