SUPPLEMENT ARTICLE

Duration of Untreated Chlamydial Genital Infection and Factors Associated with Clearance: Review of Animal Studies

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Chlamydia trachomatis **is an important cause of sexually transmitted infection that can manifest as acute cervicitis, pelvic inflammatory disease, and most commonly, chronic asymptomatic infection. The basis of this wide spectrum of manifestations and the factors that lead to clearance or chronic infection are poorly understood. We reviewed specific literature pertaining to clearance of primary genital tract infections in animal models, including mice, guinea pigs, pigs, sheep, and nonhuman primates. T helper 1 cell responses involved in cell-mediated immunity are key immune parameters that define efficient clearance in the murine and guinea pig models of chlamydial infection, which are useful for studying *C. trachomatis* clearance. However, there may be some differences between humans and other animals in innate and adaptive immune responses to chlamydial infection. Studies have suggested that differences in the induced T cell subsets and the species-specific differences in interferon γ–mediated effector mechanisms may play a significant role in these discrepancies. To close these gaps in knowledge, translational research in humans is a critical next step. However, for questions about specific mechanisms of host-pathogen interaction that cannot be answered feasibly or ethically in humans, animal models will continue to be important. Future research should include use of humanized and nonmurine models that establish prolonged infection to improve understanding of chronic human infections.**

Chlamydia trachomatis continues to be the most common bacterial cause of sexually transmitted infections in the United States; >1 million cases of *C. trachomatis* infection were reported to the Centers for Disease Control and Prevention in 2007 [1]. Reported case rates of chlamydial infection among women have more than tripled in the past 2 decades, in the era of increasing implementation of screening and early intervention strategies. The World Health Organization estimates that >90 million persons are infected worldwide [2]. Infection occurs in all socioeconomic groups and geographic areas. *C. trachomatis* genital tract infection is associated with many syndromes, including cervicitis, urethritis, endometritis, and salpingitis, and long-term sequelae include tubal infertility and ectopic pregnancy in women and urethritis, proctitis, and epididymitis in men. The infection is commonly asymptomatic and indolent and may not be cleared for several months to years [3].

A number of studies have characterized immunological responses in humans that implicate the importance of cell-mediated immunity in clearance of infection [4]. Because of the observational nature of human studies, the precise mechanism of how *C. trachomatis* establishes chronic infection and is ultimately cleared remains obscure. There are major ethical and technical

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limitations in studying both the natural course of infection and factors that may influence the outcome of these infections in humans. Thus, investigators have developed various complementary animal models in attempts to elucidate *C. trachomatis* pathogenesis [5–9].

This review summarizes the evidence from animal models pertaining to clearance of primary chlamydial infection from the genital tract. The natural course and duration of chlamydial infection in each model is discussed, followed by specific factors that have been found to contribute to its clearance. Factors associated with duration of clearance in repeat infections are related to protective immunity and will be discussed elsewhere in this supplement [10]. We also define limitations in extrapolating information obtained from animal models to humans and provide insights into how animal models may better approximate and help characterize the response to *C. trachomatis* infection in humans.

METHODS

We performed a comprehensive review of the literature based on 2 methods. A PubMed literature search using the terms "*Chlamydia trachomatis* + genital tract infection," "*Chlamydia trachomatis* + animal model," and "*Chlamydia trachomatis* + mouse model," limited to the English language, yielded >1000 articles. Relevant articles were manually selected. Articles related to pulmonary and ocular infection as well as vaccine studies were excluded, with a few exceptions. An initial list of ~200 references was compiled and then further refined. Included on the list are several excellent reviews related to this topic [9, 11–15]. References from these articles were also reviewed. Each article was carefully reviewed to gather evidence pertaining to clearance of primary infection from the genital tract. Because immunity that provides protection from future infection is reviewed separately in this supplement [10], we excluded models and findings pertaining to repeat infections.

ANIMAL MODELS OF CHLAMYDIAL INFECTION

Humans are the exclusive natural host for *C. trachomatis,* although many animals are naturally infected with other species of *Chlamydia.* Humans have been used as an "animal model" for infectious diseases caused by a number of pathogens, including sexually transmitted infections caused by *Haemophilus ducreyi* (human chancroid challenge model) [16] and *Neisseria gonorrhoeae* (urethral challenge model) [17]. However, a human chlamydial genital challenge model has been considered to be impractical and, perhaps, unsafe because of concerns about long duration of infection, risk of secondary transmission, and relative ineffectiveness of treatment.

Although other sexually transmitted infections, such as gonorrhea, syphilis, and human papillomavirus, are strongly adapted to humans and generally lack useful animal models, animal models for chlamydial genital tract infection reflect many aspects of human disease and have been extremely useful in defining factors that correlate not only with clearance but also with pathogenesis and the development of protective immunity. Animal models have been established in mice [7, 8], guinea pigs [5, 6], pigs [18], sheep [19], and nonhuman primates [20, 21]. These models often use natural pathogen-host combinations, although the route of infection may be unnatural. Thus, models differ substantially in the chlamydial species used for infection, the degree and type of pathology induced, the immune responses provoked, and the duration of infection. Obviously, no single animal species can duplicate exactly what occurs in humans, and it is important to tease out evidence from animal models that can be generalized to humans. Here, we summarize the evidence pertaining to the most commonly used mouse, guinea pig, and nonhuman primate models pertaining to duration and clearance of infection. We also highlight the discrepancies and similarities among models to address future avenues in animal and translational research.

***Mouse models using* Chlamydia muridarum.** Mouse models of chlamydial genital tract infection use common inbred mice strains and *C. muridarum* (previously known as *C. trachomatis* mouse pneumonitis strain, or MoPn). It is the most commonly used model because of the reproducible disease course, cost, and ability to manipulate immune parameters. Although earlier studies lacked uniformity of design, the model as currently used involves pretreatment with progesterone and a relatively low inoculum (median infective dose, 10–100 inclusion-forming units) delivered intravaginally. Progesterone treatment of mice increases the rate of infection and the number of chlamydiae isolated from the vaginal vault but does not affect the duration of infection [8, 22]. Studies from different laboratories have found similar kinetics of chlamydial infection and disease. Infection is monitored by swabbing the cervix via the vagina and measuring the quantity of chlamydiae (as inclusion-forming units) in cell cultures.

C. muridarum establishes infections in mice [7] with median infective doses as low as 100 inclusion-forming units. The quantity of *C. muridarum* rapidly increases during the first 7–10 days, plateaus, and rapidly decreases 3–4 weeks after infection. This relatively robust infection readily ascends to the upper genital tract and causes oviduct pathology after 5–6 weeks, making this a potentially useful model for studying the kinetics of acute lower urogenital tract infection and acute and chronic upper genital tract pathology. A single episode of infection can result in tubal infertility and fibrosis [23, 24]. The severity of infection differs depending on the mouse strain studied [25]. C3H/HeN strains are known to shed chlamydiae for a longer period than C57BL/6 strains (7 weeks vs 4 weeks) [23, 25]. This difference may be attributable to innate and acquired immune response differences that are dependent on interferon

(IFN)–γ effectors, p47 guanosine triphosphatases (GTPases), and major histocompatibility complex (MHC) class II molecules, although these were identified in nonurogenital infection models [26–28]. The kinetics of infection in male mice is less well defined but appears to last consistently for ⩾4 weeks [29].

The mouse *C. muridarum* urogenital infection model mimics human infection and immune responses in numerous ways and is very instructive in outlining the importance of broad immunological categories that contribute to chlamydial clearance. This takes advantage of an extensive mouse immunology knowledge base that has been developed over the years and the ready availability of research reagents. Chlamydial clearance is a collective function of the innate and adaptive immune responses that occur in the genital tract epithelium, resulting in infiltration of neutrophils, macrophages, dendritic cells, and T and B cells and subsequent cytokine secretion. The changes in the cell population and cytokines in the genital tract of mice are detailed in a study by Morrison and Morrison [30]. Early infiltrates in the genital tract are composed of cells of myeloid lineage, followed by a marked increase in $CD4^+$ cell count 7–10 days after infection; these form perivascular clusters of $CD4^+$ T cells that persist in genital tract tissues after resolution of the infection. $CD8^+$ T cells and $CD45R^+$ B cells are much less numerous. The involvement of numerous cell types and induction of a variety of cytokines indicate that development of immunity to chlamydial genital tract infection is a highly complex event. These candidate innate and adaptive immune factors have been evaluated further by using (1) adoptive transfer of immune cells into naive animals, (2) in vivo depletion of target cell types, and (3) knockout mice.

Collectively, these studies implicate T helper 1 (Th1) cells as having an indispensable role for resolution of chlamydial infection. Mice deficient in Th1 cells, such as nude mice [31], mice with severe combined immunodeficiency [32], T cell receptor β-chain knockout mice [33], and mice deficient in MHC-II molecules [34], either have prolonged high-level infection or fail to resolve primary infection. This is primarily an effect of $CD4^+$ T cells, because IFN-γ–producing $CD4^+$ T cell clones can resolve chronic infection in athymic mice [35, 36]. Study findings suggest that, in this process, mucosa-associated integrin receptor (α4/β7 integrin) helps $CD4^+$ T cells home to urogenital tissues [37, 38]. $CD4^{-/-}$ mice have delayed chlamydial clearance but eventually resolve infection, probably owing to the presence of non-$CD4^+$ helper cells [34].

$CD8^+$ T cell–deficient (β2M knockout) and B cell–depleted [39] or –deficient (uMT knockout) mice resolve primary infection with kinetics indistinguishable from those of wild-type mice [40]. On the other hand, chlamydia-specific $CD8^+$ T cells are generated during chlamydial infection, and adoptive transfer of these clones provides protection against genital tract infection in nude mice, albeit to a lesser degree than $CD4^+$ T cells [41]. It has been shown that antigen-specific $CD8^+$ T cells migrate to genital tract tissue inoculated with *C. trachomatis* and serve as a potential source of IFN-γ [42]. Thus, although $CD8^+$ T cells are a dispensable mechanism for resolution of primary infection in mice, they may be of greater importance in other systems. Similarly, cells involved primarily in innate immunity, such as neutrophils [43] and natural killer T (NKT) cells [44], may play limited roles in controlling early stages of infection but do not appear essential for the resolution of primary infection. This is supported by the observations that neutrophil depletion is associated with increased chlamydial shedding during the first week of infection and a relative delay in resolution and that depletion of NKT cells results in delayed resolution. It is likely that NKT cells serve as an early source of cytokines, such as IFN-γ [44].

A role for IFN-γ in clearance of primary infection has been proved by the observation of delayed infection resolution in mice in which IFN-γ has been neutralized by monoclonal antibody. In addition, passive transfer of IFN-γ to chronically infected athymic mice potently suppresses urogenital shedding of *C. muridarum* in some mice and resolves infection in others [35]. Moreover, it has been shown that, although IFN-γ knockout mice can have significantly reduced chlamydial burden in the lower genital tract, they continue to shed *C. muridarum* intermittently at low levels [32]. Although the contribution of IFN-γ to *C. muridarum* clearance in this model is clear, the impact is not as powerful as it is on *C. trachomatis,* as discussed below [32, 33, 45]. In murine cells, *C. muridarum* appears capable of evading murine-specific IFN-γ effector mechanisms that involve p47 GTPases, which may account for this difference, although this process has not been evaluated in the genital tract [46, 47]. Details on broad issues of research with the *C. muridarum* mouse model are reviewed elsewhere [9, 11–15].

***Mouse models using* C. trachomatis.** Mice can be infected urogenitally with human *C. trachomatis* serovars [8]. However, a higher inoculum is required with *C. trachomatis* than with *C. muridarum,* the peak bacterial load is 1–2 logs lower, the infection is eradicated relatively quickly [48], and pathology is mostly limited to the lower genital tract. Earlier work revealed that nude mice clear human strains of *C. trachomatis* [49] readily from the genital tract, indicating that murine innate immune responses are sufficient to clear *C. trachomatis.* These circumstances compromise the usefulness of this model for the study of acquired immunity or vaccine efficacy, but the mechanistic understanding of why *C. trachomatis* is cleared efficiently from mice is important in interpreting results of studies that use *C. muridarum.*

Studies suggest that the highly efficient clearance of *C. trachomatis* from the mouse genital tract is mediated by robust murine-specific IFN-γ–induced innate effector mechanisms [46, 47]. In contrast to the relatively modest effect of IFN-γ

on *C. muridarum,* several groups have shown that *C. trachomatis* infection of IFN-γ or IFN-γ receptor knockout mice results in a more pronounced delay in clearance of this species, compared with similar experiments in the *C. muridarum* model [45, 50]. The IFN-γ effector mechanisms in humans are primarily thought to involve indoleamine dioxygenase (IDO) activation that depletes intracellular pools of tryptophan, which starves human serovars of *C. trachomatis* of an essential amino acid. The end effect of this at high IFN-γ concentrations can be cidal, whereas at lower concentrations, the result is to drive the organism into a viable but nonreplicating "persistent" form [51, 52]. Of interest, mice do not appear to induce IDO in response to chlamydial infection but instead have redundant mechanisms that involve IFN-γ–inducible effectors inducing nitric oxide synthase, p47 GTPases, and perhaps other mechanisms that appear to have bactericidal effects on the organism in cell culture [53]. However, low-level infection appears to continue in mice, because chlamydial nucleic acids (major outer membrane protein DNA and 16S RNA) can be detected for very long periods after culture resolution of infection [54]. Shedding of *C. muridarum* (albeit briefly and in low numbers) can be reactivated for up to 3–4 weeks after culture-proven resolution of infection [22, 54]. Thus, although different endline IFN-γ–mediated mechanisms may be at work in humans and mice, persistent and chronic low-grade infection appears to occur in both species, and in both species, it is plausible that persistence versus clearance is driven by IFN-γ responses.

Guinea pig models. Guinea pigs can be infected urogenitally with *Chlamydia caviae* (formerly *Chlamydia psittaci* guinea pig inclusion conjunctivitis strain), which causes conjunctivitis in the natural infection [55, 56]. The guinea pig genital tract infection model was one of the first to be established [9]. Inoculation with *C. caviae* in the lower genital tract causes a self-limited infection with a duration of 3–4 weeks that involves the squamous epithelium of the ectocervix and squamocolumnar junction. Ascending genital tract infection of the oviducts occurs in ~80% of infected guinea pigs [57]. This model has several unique advantages over other animal models. Infections can be established in male animals, thus providing the opportunity to model sexual transmission [56]. In addition, vertical transmission from mothers to newborn pups [6] occurs in this model. Guinea pigs also have a long estrous phase (17 vs 4–5 days in mice) comparable to that in humans, making this a more appropriate model to study hormonal influences on pathogenesis. Oral contraceptive agents, consisting of mestranol and norethynodrel, enhance ascending infections in female guinea pigs and prolong duration of infection [58, 59].

The immune responses seen in guinea pigs have also been studied in detail, and findings are in some ways similar to but in other ways different from what has been shown in murine models. Early infection (before or on day 5) is characterized by an influx of polymorphonuclear cells to the epithelium and mononuclear cells by day 10 [60]. Involvement of T cells in guinea pigs has been evaluated using anti-thymocyte serum [61], which results in a depletion of T cells only in the peripheral circulation. This leads to a prolonged low-level infection throughout the anti-thymocyte treatment course, compatible with findings in T cell–depleted mice. Cell populations associated with resolution of infection involve more $CD8^+$ T cells than in mice [62], similar to what has been seen in nonhuman primates [63] and humans [64]. In contrast to mice, in which B cell depletion had no role in resolving primary infection, decreased antibody production has been found to correlate with delayed clearance of primary infection in guinea pigs treated with cytotoxic agents or estradiol [65, 66].

A problem with the guinea pig model is the limited available reagents with which to study immune responses in this species. Considering that guinea pigs are used extensively in other infection models, there is hope that more reagents will be made available and this problem can be overcome.

Nonhuman primate models. Nonhuman primates, including the marmoset, grivet, and pigtailed macaque, have been used as models for *C. trachomatis* genital tract infection for decades and would intuitively be the closest surrogate models for humans. Infection in grivet monkeys elicited acute salpingitis after inoculation into either the fallopian tube directly or the uterine cavity [67]. Studies on the mode of spread of chlamydial infection continued in the grivet model [68]. Marmosets were studied as a model for ascending chlamydial infection; cervicitis resulted after intravaginal inoculation, but upper tract disease was minimal [69].

Pigtailed macaques were first used in the early 1980s as a model for chlamydial genital disease. The advantages of this primate model include the animal's size, relatively quiet temperament, and well-characterized menstrual cycle. The normal anatomy and physiology of the macaque reproductive tract are remarkably similar to those in humans. Pigtailed macaques are naturally susceptible to cervical infection with human biovars of *C. trachomatis,* which may be cultured from the cervix for up to 15 weeks [70] after primary inoculation. The kinetics of infection are distinct from those in mouse or guinea pig models. Shedding from the cervical os is intermittent, and the peak of the infection may be delayed for months. Repeated cervical inoculations cause salpingitis and peritubal adhesions, although intratubal challenge increases the severity of upper tract disease [71]. These observations suggest that macaques develop a chronic indolent infection similar to that documented in humans. In parallel with the in situ pigtailed macaque challenge studies, an autotransplant tissue model was developed in the pigtailed macaque. This model allows for multiple samplings of infected salpinx tissue over time and requires minimal surgical intervention [21].

Initial immune responses in nonhuman primates are characterized by early polymorphonuclear cells in the endocervix [69], followed by mononuclear lymphocytic infiltrates similar to those in other animal models [20]. Humoral response, as evidenced by serum and cervical secretory antibody production, also has been documented in macaques [20]. Van Voorhis et al [63] showed that, after a single inoculation, cytokine gene expression profiles consistent with Th1-like responses occur. However, the predominant cell infiltrate after single or repeated *C. trachomatis* inoculation consists of $CD8^+$ T cells rather than $CD4^+$ T cells or B cells and suggests a fundamental difference between rodents and primates with regard to the T cell subset responses to chlamydial infection [72]. Data from genetic studies of variations in the outcome of infection in macaque monkeys indicate the association of pathology with genetic polymorphism in the MHC-I molecule and support the aforementioned findings [73].

Other animal models. Evidence for chronic chlamydial infection or persistence in alternative large animal models holds promise, but these models are much less well studied than murine, guinea pig, and nonhuman primate models. Pigs are naturally infected by *Chlamydia suis* and *Chlamydia abortus.* Although these species cause natural enteric and lung infections in pigs, experimental models of genital tract infection also have been developed using human *C. trachomatis* serovar E strains [74]. *C. trachomatis* establishes infection in the epithelium of the endocervix and uterus in female pigs (gilts), resulting in an erythematous vulva along with an elevated temperature, suggesting a systemic response to infection. Ascending infection has been documented, with chlamydial shedding detected for up to 21 days. Although the observations with this model are somewhat limited, in enteric models of *C. suis* infection, chlamydiae can be observed in tissues as aberrant forms, suggesting that *C. suis* may establish persistent infection in pigs [75].

The pig model also has potential use in evaluating hormonal responses. In humans, the use of oral contraceptives is also known to be associated with increased rates of infection [76], and the stage of the estrous cycle affects growth of chlamydiae in human cells [77]. Likewise, the rate of infectivity of primary cells obtained from pigs was greatest when cells were obtained during the estrogen-dominant phase [78]. *C. abortus* causes abortion and stillbirth in sheep and can persist in the females for >1 year after they give birth [19, 79]. Although this model is used primarily to understand the pathogenesis of abortion, persistent infection of the reproductive tract has been documented. Very little is known about the immune responses responsible for resolving infection in this model.

USEFULNESS OF ANIMAL MODELS IN STUDYING HUMAN *C. TRACHOMATIS* GENITAL TRACT INFECTION

The ultimate goal of research is to determine effective public health interventions to control chlamydial genital tract infections; an understanding of basic chlamydia-host biology can shed light on whether to implement a number of public health strategies. Toward this end, one role of animal models is to guide the development of safe and effective vaccines. This, in turn, requires understanding of the factors that influence clearance of infection. Studies in humans and animals have found that immunological parameters, genetic background, and hormonal influences affect clearance of chlamydial infection in either system. However, drawing parallels from animal models to humans remains a challenge, and we must now ask difficult questions concerning what we can and cannot apply to humans and how we can design future animal studies to maximize translational value.

Despite the differences among models, a consistent single major conclusion can be drawn from animal studies that probably has significance for human infection [4]: elicitation of T cell–mediated immunity is important for clearance of the organism. Studies using mouse models have identified MHC-II antigen processing and $CD4^+$ T cell/Th1 cell responses mediated by IFN-γ as central to chlamydial clearance in primary infection. Studies in guinea pigs further implicate the role of T cells and, possibly, antibodies. Although it is difficult to determine what induces clearance in nonhuman primates, immunological correlates of clearance suggest involvement of T cells and Th1 cytokines. Finally, in humans, chlamydial genital tract infection in women results in secretion of local immunoglobulin A and G responses and infiltration by lymphocytes that have proliferative responses against *C. trachomatis,* indicating that both humoral and cell-mediated immune responses are induced by infection and ultimately result in clearance [4]. This concept of eliciting a proper Th1-mediated T cell response is central in the current design of vaccines against chlamydia.

However, there are a number of unresolved issues. A major difference vis-à-vis human infection is the variability in the kinetics of chlamydial growth and the duration of primary infection in these animal models. In mice or guinea pigs, detection of viable chlamydiae from the lower urogenital tract is limited to the first 3–4 weeks of relatively robust infection course, although mice continue to shed chlamydial nucleic acids for months, and small numbers of viable organisms can be recovered if mice are immunosuppressed [54]. Indolent intermittent shedding of viable *C. trachomatis* has been documented for up to 15 weeks in primates [70], and *C. psittaci* shedding has been documented for up to 1 year in sheep [79]. Studies in humans suggest that half of infected women may continue to be infected beyond a year [3]. These differences are an in-

dication that the course of chlamydial infection differs to various degrees depending on the animal host, the chlamydia species used, and the anatomic site of infection for each model. They also pose a challenge both for interpreting what we learn from animal models and for developing new research questions.

At least 2 clear differences in immune responses may account for the observed differences in the duration of infection. In guinea pigs and monkeys, $CD8^+$ T cells are more abundant than $CD4^+$ T cells, which are associated with resolution of infection in mice. Some data suggest that $CD8^+$ T cells play some role at least in the initial phase of infection resolution, whereas in mice, they are dispensable. Both $CD4^+$ and $CD8^+$ cells are recruited to the endocervix during *C. trachomatis* infection in humans, and the proportion appears to be situational, based on clinical presentation [80]. In this study, higher organism load in cervical swab samples from women with chlamydial infection was associated with an increased number of local $CD4^+$ cells [80]. Because of the cross-sectional nature of the study, it is unclear whether this represents recruitment of protective cells in response to increasing chlamydial load or a relative ineffectiveness of $CD4^+$ T cells in human infection. The basis for the differences in T cell subsets and whether they reflect efficiency of clearance are still unknown. A number of studies and reviews have addressed the differences in IFN-γ effector mechanisms in mice and humans [46]. Mice induce effectors that include p47 GTPases, which appear to have sterilizing immunity against a variety of intracellular organisms. This defense system seems almost completely absent in humans, in whom the primary response seems to be tryptophan starvation by IDO, which would cause a persistent state of infection [81]. This difference could explain the discrepancies between mice and humans in the kinetics of chlamydial growth and shedding, although it also has not been validated in vivo.

Two possible avenues in animal research may address these issues. One approach is to exploit nonmurine models, and the other is to generate "humanized" animal models. Currently, there is little mechanistic understanding of the immune responses in models other than mice and guinea pigs. For example, do nonhuman primates induce IDO in response to IFN-γ and induce a state of tryptophan starvation? The use of class I matched animals used in human immunodeficiency virus (HIV) or simian immunodeficiency virus research [82] may also help in exploring the basis of the $CD8^+$ T cell–predominant responses observed in primates. These models may prove to have better correlates to human responses and may help explain the aforementioned differences.

Decades of research in the field of humanized mice have led to the creation of mice that allow engraftment of human tissue, ranging from hematopoietic cells to skin, enabling infection of mice with agents that have high species-specific tropism, such as HIV and varicella-zoster virus [83, 84]. Although it is still impractical to study the pathogenesis of *C. trachomatis* directly in mice owing to the barrier in species-specific innate immunity, such advances may allow this. With current capabilities, mouse models will continue to be useful in defining specific molecular mechanisms. In addition, as mentioned above, removal of murine-specific effectors promotes chronic infection [54], suggesting that this is a potential strategy in studying chronic infection. For example, we could generate mice that are deficient in murine IFN-γ effectors but instead express IDO, a predominant IFN-γ response mechanism in human cells, to address the specific question of in vivo persistence and interaction of genital strains of *C. trachomatis* that possess tryptophan synthase [81].

Although research has stressed identification of immune factors related to chlamydial clearance, individual variability in disease severity in human populations may also be attributable to the complex interaction of the host genetic factors and environmental factors. Animal models can be used to model individual factors that influence effective public health strategies. For example, a relatively simple mouse model using antibiotics revealed that early antibiotic therapy in mice hinders the development of effective immunity [85]; a parallel process was hypothesized to explain an increased incidence of repeat infection in a human population after the implementation of screening and early treatment (the "arrested immunity" hypothesis) [86]. Genetic differences in mouse strains and macaques influence resolution of infection and are likely to be attributable to polymorphisms in immune-related genes. Although genetic studies in animal models may not provide direct correlates to humans, they define novel sets of genes and immune mechanisms that may be important in clearance of chlamydia and can be explored in translational human studies. They also help define the similarities and differences between animal models and humans and, thereby, the usefulness of each model described thus far. Genetic polymorphisms may also serve as tractable targets for human research because of the difficulty in assessing immune responses during a natural infection. In this context, animal models may guide the design of clinical studies.

Such creative use of animal models will build on this valuable body of knowledge but will need to be carefully designed to address differences between animals and humans. At the same time, we will need to take the difficult steps necessary to promote more translational research and apply what we have already learned from animal research to humans.

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