

Protective Immunity to Chlamydial Genital Infection: Evidence from Animal Studies

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In all animal models for chlamydial infection, there is strong evidence for immunity to reinfection; however, immunity is only complete (ie, preventing infection) in the short term. In the long term, animals are only partially immune (ie, they can be reinfected, but infections are usually abbreviated and less intense than the primary infection). This review will target the mechanisms responsible for long-term versus short-term immunity and explore the roles of various components of the host response in immunity to chlamydial genital infection.

An important question about the natural history of *Chlamydia trachomatis* genital infection, with implications for both chlamydia control programs and vaccine development, is whether immunity to reinfection develops naturally; if so, how long does that immunity last after a natural infection, what are the effector mechanisms, and are there any confounding issues, such as an enhanced pathologic response? If immunity develops, other important considerations for public health programs include how long immunity takes to develop and whether treatment at different times may affect its development. In addition, understanding the various parameters of natural immunity gives us a target to either duplicate or surpass with an appropriate vaccine candidate and immunization regimen. In this section, we will confine our discussion to what has been learned about immunity to reinfection in the 3 major animal models of chlamydial genital tract disease—nonhuman primates, mice, and guinea pigs—and extrapolate to

humans when appropriate. In addition, because some very relevant studies on the protective immune response to *C. trachomatis* have been performed in the nonhuman primate model of trachoma, a discussion of that model has also been included. We have insufficient space to define and characterize the models in detail, but extensive reviews are available [1–6].

Although the purpose of using animal models is to be able to extrapolate to humans, there are significant differences between human or nonhuman primate infections with *C. trachomatis* and infections of the mouse and guinea pig with *Chlamydia muridarum* and *Chlamydia caviae*, respectively. The latter agents clearly have different natural histories; *C. muridarum* and *C. caviae* are gut parasites for their natural hosts and have only a single serovar, whereas *C. trachomatis* is specific to human ocular and genital infections and has 18 serovars. It is not known how these differences evolved, but they prevent studies addressing the implications of this phenomenon in the mouse and guinea pig models.

When considering protective immunity in the context of chlamydial infection, it is important to address immunity with respect to not only clearance of the organism but also prevention of disease. This is of particular importance in chlamydial infection, because there are substantial data indicating an important role for the host response in the development of pathology. It is entirely possible that immunity to the organism may exist; however, introduction of the organism, even in an aborted or abbreviated infection, may elicit a

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pathologic response. Nevertheless, in this article, only immunity to the organism will be addressed. Mechanisms of pathogenesis and pathology and their relationship to the immune response are discussed elsewhere [7].

IMMUNITY TO CHLAMYDIAL GENITAL INFECTION: EVIDENCE FROM ANIMAL MODELS

Perhaps the most important question is whether immunity to chlamydial genital infection develops after a primary infection. The answer to that question is a resounding “yes.” In all evaluated animal models of genital infection, including the mouse [8, 9], guinea pig [10], macaque [11], and marmoset [12], immunity to reinfection develops. However, although immunity develops, the extent of that immunity needs to be qualified. Essentially, there are 2 levels of immunity: (1) complete immunity, in which no organisms can be isolated or detected at the site of inoculation after reinoculation, and (2) partial immunity, in which organisms can be isolated or detected at the site of reinoculation but the infection course is abbreviated and/or the peak level of infection is lower than that during a primary infection.

Studies in animal models have shown that whether an animal displays complete versus partial immunity depends on the period from resolution of the primary infection in the absence of antimicrobial treatment to reinfection. Complete immunity to reinfection has been demonstrated in both mouse and guinea pig models of chlamydial genital infection, but reinfection must occur within a short period after resolution (1–3 months). BALB/c mice infected with *C. muridarum* were found to be resistant 42–60 days after the primary genital infection or ~3 weeks after resolution of the initial infection; however, some could be reinfected when challenged at 100 days, and 100% were infected when challenged at or after 150 days [8, 9]. Female guinea pigs infected genitally with *C. caviae* were completely immune 30 days after a primary infection or 1–2 weeks after resolution, but all animals became infected when challenged 75 days after the primary infection [10]. Of interest, a higher percentage of male guinea pigs had complete immunity for a longer period, compared with female guinea pigs [13].

In contrast, mice and guinea pigs maintain partial immunity for exceedingly long periods. When challenged 200 days after a primary infection, mice still had abbreviated courses of infection, with lower peak numbers of chlamydiae [9]. Assuming that a mouse lives ~2 years, that period represents >25% of its life span. Batteiger and Rank [14] reinoculated guinea pigs 825 days after a primary infection and found that the peak level of infection in all of the animals was still decreased, compared with the primary infection. Although the infections were also shortened, they were not as short as reinfections 75–150 days

after a primary infection. In terms of a guinea pig’s lifespan, these data indicate that partial immunity remained present for 50%–60% of the animal’s life. These studies suggest that a single infection can elicit long-term immunity, albeit only partial immunity, whereas complete immunity is present only for short periods. It should be noted that, although the animals may have a degree of protection with respect to the organism, this does not mean that they are protected against upper tract disease and tubal obstruction. In fact, these may have developed as a consequence of a primary infection and could be either exacerbated if already present or newly produced after a challenge infection if they did not develop initially [15–17].

Protective immunity appears to develop rather quickly after infection, with both antibody and cell-mediated immunity detectable within 10–14 days after infection in both mice and guinea pigs [8, 14, 18]. When the immune response is interrupted by the elimination of organisms by antibiotic treatment at various intervals after infection, the data indicate that mice must be infected for >10 days for optimal immunity to develop [19]. Similar results were observed in guinea pigs, because clearance of infection by antibiotics \leq 9 days after infection prevented immunity from developing (N. Schmucker and R.G.R., unpublished data). Although immunity arises very quickly in animal models, this does not seem to be the case in humans, and the reason for this difference is an obvious point of concern.

In real life, individuals are not exposed to chlamydiae only at a single defined time, as in animal studies, but are often repeatedly inoculated. This raises the question of the impact of repeated inoculations on the nature and quality of immunity in those individuals. It is well known that, in trachoma, in which individuals are being repeatedly inoculated, it is often difficult to isolate the organism, even in the presence of a pathologic response. This suggests the presence of a protective immune response, albeit one that may be eliciting a pathologic response analogous to a delayed-type hypersensitivity response, in which only a small amount of antigen can elicit a strong pathologic response [20, 21]. These observations have also been supported by studies in the nonhuman primate model of trachoma [22]. Does a similar phenomenon occur in genital tract infections? Data obtained from a longitudinal study involving female sex workers showed increased protection against reinfection with genotypes with which the women were previously infected [23]. These results may indicate that repeated infection increases the level of protective immunity.

There is also some evidence in humans to indicate that older individuals become more resistant to infection than adolescents [24]. However, even when frequency of exposure was controlled for, individuals aged 20–30 years still have an apparently higher level of resistance than do adolescents, suggesting that the dif-

ference in susceptibility may be associated more with age than with frequent exposure.

Only a single study has been performed in an animal model of genital tract infection to address this scenario of multiple exposures. Rank et al [10] infected guinea pigs intravaginally with *C. caviae* and then challenged different groups 30, 75, or 150 days later. The majority of the animals challenged at 30 days after the primary infection were completely immune to reinfection, whereas the guinea pigs challenged 75 or 150 days after initial infection became reinfected, although the infection course was less intense and abbreviated. To determine whether the interval between repeated challenges was a critical factor or whether 2 infections elicited stronger immunity than only a single infection, the animals reinfected 150 days after the primary infection were reinoculated intravaginally 30 days later, animals reinfected at 75 days were reinoculated 75 days later, and animals reinfected at 30 days were reinoculated 150 days later. Of interest, the only group demonstrating complete immunity was the group challenged 30 days after the previous inoculation. All other groups became reinfected, and there was no difference in the kinetics between the second reinfection and the first reinfection. Therefore, there was no evidence of enhanced immunity with multiple challenges, and the critical factor with regard to whether the animals had complete or partial immunity was the time between challenges. As discussed below (see "Explanation of Short-Term Immunity"), these data do make sense in light of the potential mechanism, but nevertheless, the findings are not very encouraging with respect to eliciting long-term complete immunity through vaccination.

Of note, in virtually all studies on protective immunity, animals are always inoculated artificially, not through the natural route, which is sexual transmission. The only model available to evaluate sexual transmission is the guinea pig model, in which males can be infected and will transmit the infection to females [25, 26]. In the only study in which guinea pigs were infected sexually and then given an artificial challenge infection, the animals proved to be immune to reinfection, indicating—not surprisingly—that sexual transmission elicits a protective immune response [27].

IMMUNE EFFECTOR MECHANISMS OF PROTECTIVE IMMUNITY IN ANIMAL MODELS OF GENITAL INFECTION

In understanding the mechanisms of protective immunity, we must consider not only how the host response is able to control and eliminate the organism but also whether there are different mechanisms operative during the short period when animals exhibit complete immunity than during the extended period of partial immunity. If the mechanisms are not different in complete and partial immunity, why does complete immunity cease in a relatively short time? To date, animal studies have

demonstrated that natural infection elicits the highest level of protective immunity, although long-term complete immunity does not develop. An understanding of why complete immunity does not develop as a result of natural infection should give us insights into strategies necessary for the development of an effective vaccine.

Role of innate response. In primary infections and reinfections, the first host response is an acute inflammatory or polymorphonuclear leukocyte response, probably initiated by activation of the Toll-like receptor pathway and by other still-undefined mechanisms. In vitro studies have shown that infection of host cells by chlamydiae elicits chemokine and cytokine production within the first 24 h [28, 29]. However, although studies indicate that the initial acute inflammatory response is important in controlling the primary infection until the adaptive response can be activated [30], there are virtually no data on the role of the acute inflammatory response in protective immunity. Nevertheless, it would stand to reason that, at least in partial immunity, this response may play a role in controlling infection, because any infection cycle in a host cell will result in chemokine and cytokine production. Another important component of the innate response is the natural killer (NK) cell, which can be found in the mouse genital tract within 12 h after *C. muridarum* infection and can help control chlamydial infection through the production of interferon (IFN)- γ [31]. Again, although it is likely that NK cells enter the genital tract in response to reinfection, there are no published data to confirm such a role.

Role of antibody. Several studies in both mouse and guinea pig models have addressed the mechanism(s) of protective immunity, although there are distinct differences between the 2 models. Both animals produce a strong antibody response as a result of chlamydial genital infection that is long lasting in serum but relatively short lived in genital secretions [9, 14]. In guinea pigs infected intravaginally with *C. caviae*, antibody appears to be essential for immunity to reinfection. When guinea pigs are treated with cyclophosphamide at 9-day intervals, antibody does not develop and animals are unable to resolve infection, even though the cell-mediated immune response is intact [32]. If the infection in antibody-deficient animals is cured by treatment with tetracycline, they still remain susceptible to reinfection. In fact, they are unable to resolve the challenge infection, and the level of the challenge infection is not reduced in intensity [33]. Of interest, it was observed that some animals recrudesced without being challenged, suggesting that either the tetracycline did not completely cure the animals or a drug-resistant variant was selected. Regardless, it was clear that antibody was essential to control or eliminate the infection in guinea pigs.

Intuitively, the local genital tract antibody response, particularly secretory immunoglobulin (Ig) A, would be expected to

be the primary source of antibody. Supporting this premise, treatment of guinea pigs with estradiol increased the intensity and duration of chlamydial genital infection in the guinea pig, and this increase in the duration of infection was associated with a delay in the detection of antibody in genital tract secretions [34]. However, it was later reported that, when guinea pigs were passively immunized with the gamma globulin fraction of convalescent serum, IgG titers in genital secretions that were comparable to those in natural infection could be attained [35]. When challenged, passively immunized animals had significantly lower levels of chlamydiae in the genital tract, although the course of the infection was no shorter than that in control animals. These data suggested that the primary source of antibody in the female genital tract is serum that enters the genital tract through the process of transudation. Indeed, the dominant isotype of immunoglobulin in the female genital tract is IgG, not IgA [36]. This is supported by observations in both guinea pig and mice that IgA decreases to low or undetectable levels in genital tract secretions by ~50 days after infection [9, 14]. In contrast, specific antibodies to chlamydiae can be detected for ≥ 825 days in guinea pig genital secretions and for >200 days in mouse genital tract secretions, over which time animals demonstrate partial immunity [14].

The requirement for antibody in the mouse *C. muridarum* model is somewhat less obvious. Mice made B cell deficient by treatment with anti- μ antibody are perfectly capable of resolving a primary infection and are immune to reinfection, which suggests that the cell-mediated immune response is the critical component of the host response in the mouse [18]. Similarly, B cell knockout mice also can resolve a primary infection equivalent to controls and are resistant to reinfection, although the challenge infection does take marginally longer to resolve [37]. Of interest, when mice with resolved chlamydial genital infection were treated with either anti-CD4 or anti-CD8 antibodies and then reinfected, the infection was prolonged in mice treated only with anti-CD4 antibody. However, when immune B cell-deficient mice were treated with anti-CD4 antibody, the challenge infection did not resolve, indicating that both antibody and CD4⁺ cells were essential for immunity to reinfection [38]. Furthermore, when B cell-deficient mice deprived of CD4⁺ cells were passively immunized with immune serum, the challenge infection resolved [39, 40]. That passive immunization through an intraperitoneal route successfully cures the infection in mice also indicates that the source of protective antibody is probably systemic rather than local in the genital tract. Therefore, the data for mice also strongly support an important role for antibody in resistance to reinfection.

There are no in vivo data available to discern the exact mechanism by which antibody causes resolution of an infection. Nevertheless, one could surmise that the mechanism is probably one of neutralization, such that antibody binds to elementary

bodies and prevents them from either entering host cells or transforming into reticulate bodies in the cell. There is ample in vitro evidence to demonstrate that antibody can block chlamydial infection via neutralization [41, 42]. Alternatively, antibody may opsonize elementary bodies and enhance uptake by phagocytes [43]. This mechanism has also been demonstrated in vitro. It is likely that both mechanisms are operative in vivo.

Role of cell-mediated immunity. It is very clear from both mouse and guinea pig animal models of chlamydial genital infection that cell-mediated immunity is required for both resolution of a primary infection and protective immunity. Ramsey et al [18] first reported that, when B cell-deficient mice were infected, they were able to resolve the infection as effectively as were immunologically intact control mice. Of importance, they found that, even in the absence of antibody, the mice were immune to reinfection, which indicates that T cells alone could provide protective immunity. Su et al [37] found a similar phenomenon in B cell knockout mice. Moreover, when B cell-deficient mice were treated with anti-CD4 antibodies but not anti-CD8 antibodies, resolution of the infection could be prevented, indicating that CD4⁺ T cells are also essential for protective immunity in the mouse [38]. As in resolution of a primary infection, it does not appear that CD8⁺ cells are essential for immunity to reinfection.

Similar to findings in mice, when guinea pigs in which *C. caviae* genital infection had resolved were treated with antithymocyte serum before and during challenge infection either 30 or 75 days after infection, all of them became reinfected [44]. It was not surprising that they became reinfected, particularly when they were challenged on day 75, but the infection did not resolve for as long as the animals continued to receive the antithymocyte serum treatment. Moreover, the level of the challenge infection was very low, compared with a primary infection or reinfection in B cell-deficient guinea pigs. Thus, as in mice, guinea pigs require both humoral and cell-mediated immune responses for complete or partial immunity to reinfection. We can hypothesize that antibody is necessary to reduce the infectious load in the genital tract and that CD4⁺ T cells are essential for final elimination of the infection. Although it has not been evaluated, the protective CD4⁺ T cell response in reinfection in the guinea pig model is likely to be a Th1 response, as in the primary infection [45], and the mechanism is probably mediated by IFN- γ .

Explanation of short-term immunity. As stated above, immunity to chlamydial infection is complete early after resolution of infection but then becomes partial as the interval increases between resolution of infection and a second infection [10]. An important difference between the antibody and cell-mediated immune response is that IgG antibody persists in the genital tract through the constant replenishment from serum

antibody through transudation [14]; in contrast, T cells remain in the genital tract only as long as chlamydial antigen is present. Igiertseme and Rank [46] assessed the local T cell proliferative response in the guinea pig cervix at varying times after infection and observed that the antigen-specific T cell response reached peak levels at the time that infection was resolved, then decreased rapidly by day 30, and was at baseline levels by day 75. Of interest, although most of the animals demonstrated complete immunity at 30 days, the animals became susceptible to reinfection as the local T cell response decreased to baseline levels. Nevertheless, the peak level of the challenge infection was significantly decreased, most likely because of the presence of antibody. The duration of the challenge infection was also abbreviated, suggesting that there was a rapid anamnestic T cell response.

The rapid decrease in CD4⁺ T cell count and vascular cell adhesion molecule 1 and mucosal addressin cell adhesion molecule 1 expression was also observed in the mouse model of *C. muridarum* genital infection [47]. The decrease in CD4⁺ T cell count in the genital tract corresponded to the time when mice lost complete immunity and were only partially immune [9]. Similarly, when a fluorescence-labeled protective CD4⁺ Th1 T cell clone was adoptively transferred into normal mice at varying times after *C. muridarum* genital infection, the greatest number of cells homed to the genital tract 7 days after infection, indicating that the chemokine gradient had already developed within 1 week after infection [48]. By day 35, after resolution of the infection, the T cell clone could no longer be found in the genital tract, supporting the hypothesis that clearance of the infection removed the stimulus for eliciting the chemokine response. Therefore, complete immunity occurs only for a short period after resolution of the infection, when CD4⁺ T cells are still present in the genital tract. When chlamydiae are no longer present, there is no longer a mechanism for producing the appropriate chemokines and cytokines to elicit expression of addressins and to develop a chemotactic gradient; consequently, T cells leave the local site.

However, at the time of reinfection, when T cells have left the genital tract, chlamydiae infect target cells and elicit a chemokine and cytokine response through activation of signal transduction pathways, resulting in the expression of addressins on endothelial cells and the formation of a chemotactic gradient to attract circulating T cells to the local site. Clearly, this can occur quickly but not instantly; thus, chlamydiae would have the opportunity to initiate infection before activated T cells finally enter the site and resolve the infection. Kelly and Rank [47] reported that, at the time of reinfection, CD4⁺ T cells already had attained substantially higher levels by day 7 than in a primary infection, indicating a vigorous anamnestic T cell response. In contrast, there was not a strong anamnestic response with CD8⁺ T cells.

This concept of complete versus partial immunity suggests that the development of a vaccine that elicits long-term complete or sterilizing immunity is going to be extremely difficult. It is not difficult to conceive of a vaccine eliciting a strong antibody response that could reduce the level of infection in the genital tract for a long period, but eliciting a long-term resident T cell response in the genital tract is highly unlikely and not even desirable. Although a vaccine that induces sterilizing immunity is desirable, one that could reduce the bacterial burden via antibody and prime the individual for a strong and rapid anamnestic T cell response to eliminate the infection before the organism can ascend the genital tract would be more than satisfactory, because prevention of tubal obstruction is the most important objective of a vaccine.

LESSONS FROM NONHUMAN PRIMATE STUDIES ON IMMUNITY TO TRACHOMA

Nonhuman primate models for chlamydial disease have been in use for >50 years and have yielded a great deal of information on both genital and ocular infections. A major advantage in the use of nonhuman primates is that human chlamydial biovars can be used, and for trachoma, the ocular biovars have been routinely used. A disadvantage to the use of monkey models has been their high cost, which limited the numbers of animals in experiments, and the outbred nature of the animals, which introduced greater immunologic variation in results. Although nonhuman primate models for genital tract infection have been extensively used, the studies have focused on the pathogenesis of infection and resolution of the primary infection but have not addressed naturally developed protective immunity. In the context of protective immunity in a physiologically relevant model, perhaps the most information has emerged from the monkey model for trachoma. Initially, the trachoma studies in nonhuman primates involved detailed and numerous challenge studies in animals immunized with a variety of chlamydial preparations and adjuvants. Although not relevant to the topic of this article, which deals with protective immunity developed after natural infection, there was much information gained regarding serovar specificity and duration of immunity.

However, important work on protective immunity was published in reports of several studies by Taylor et al [22, 49], who used *Macaca* infected in the conjunctiva with the Bohr strain of serovar E. In this model, animals were infected multiple times to simulate what actually occurs in humans. After 8 weeks of weekly ocular reinfections, the number of culture-positive samples decreased, and the inflammatory index was reduced significantly, compared with the follicular index. In another experiment, the animals were reinfected weekly for 40 weeks [22]. During this time, clinical disease scores remained high rather than decreasing, as they ultimately would after a single infectious challenge. In contrast, after 6–8 weeks, all animals

became culture negative regardless of repeated challenges. These data suggest that protective immunity indeed developed, but how long the animals remained protected was not determined.

Immune effectors involved. Antibody responses were represented by all subclasses, IgG, IgM, and IgA. IgG and IgA levels trended upward when resolution of infection occurred, which suggests that the latter antibodies could be important in trachoma and in ocular chlamydial infections. Most studies in monkeys focused on serologic responses in serum and tears or secretions, and infections were limited to homologous rechallenge. An early study by Caldwell et al [50] showed longitudinal development of tear IgA and IgG after B/TW-5 ocular infection. Another study of repeated infections after >40 weeks showed similar clinical scores for BOUR (E serovar) and HAR-1 (A serovar), although E serovar induced higher serologic responses [22]. Antibody responses were found to be long-lasting in the trachoma model, particularly IgG in tears and serum. If monkeys were repeatedly reinfected, both tear IgG and IgA remained positive at high titers. This observation contrasted with that in patients with trachoma, in whom tear IgA but not tear IgG specific for major outer membrane protein (MOMP), heat-shock protein 60 (hsp60), and whole elementary bodies remained high in patients with trachomatous scarring [51].

It was also observed that, if protective immunity develops after a single infection, inflammation decreases rapidly, even while the follicular index is maintained. If the animal is re-challenged, inflammation increases again; histologically, a mononuclear infiltrate increases with each rechallenge [52]. Additional studies of repeated infections in the same cynomolgus monkey model demonstrated that weekly repeated infections increased the CD4⁺ and CD8⁺ T cell counts in infected, inflamed conjunctivae by ≥ 2 -fold over those seen with single challenges. In all cases, CD8⁺ T cell counts were much higher than CD4⁺ T cell counts [52]. Of interest, conjunctival tissue samples obtained during wedge surgery in patients with trichiasis had a higher CD4⁺ T cell count than CD8⁺ T cell count [53]. It has not been determined whether these differences reflect the disease stage of late trachomatous scarring in patients, compared with an earlier disease stage in monkeys, or some other fundamental difference between clinical disease in humans and nonhuman primates. Of interest was the observation that the cynomolgus monkey model showed evidence of T suppressor or cytotoxic CD8⁺ T cells [54]. These studies preceded studies of other potential immunoregulatory T cells in immunity to chlamydial infections, and their findings suggested a possible mechanism for the decline of the cellular response after resolution of infection.

Local immune responses to ocular chlamydial infection. Evidence for development of local versus systemic immunity during ocular chlamydial infections was obtained from the cynomolgus monkey models, and this recapitulated earlier studies

in owl and Taiwan monkeys [49, 55, 56]. Initially lymphocytes were isolated from conjunctival biopsy specimens during active infection. Both T and B cell proliferative responses were induced by exposure to elementary bodies, with conjunctival responses being much stronger than those detected for peripheral blood mononuclear cells [57]. *C. trachomatis*-specific antibodies were secreted during in vitro culture of conjunctival lymphocytes with elementary bodies, and these observations were congruent with antibodies detected in tears. Similar results were obtained with conjunctival lymphocytes isolated after topical ocular challenge of immune animals with Triton extract hsp60 antigen (which induced rapid follicular and inflammatory responses in direct fluorescent antibody and culture-negative conjunctivae). These results were extended in additional studies that compared local and regional immune responses after ocular infection in cynomolgus monkeys. Draining lymph node samples were collected and compared by limiting dilution with respect to the frequencies of *C. trachomatis*-specific T cells in conjunctivae, distant lymph nodes, and peripheral blood lymphocytes. The T cell frequencies in the draining lymph nodes were 5–10-fold higher than those for peripheral blood lymphocytes. In contrast, distant lymph nodes had similar responses to the peripheral blood lymphocytes [58, 59]. Similarly, the frequencies of *C. trachomatis*-specific B cells determined by enzyme-linked immunospot assays paralleled those seen for T cells [59]. These studies were important because they demonstrated, for the first time in a chlamydial infection model, the varied frequencies of specific T and B cells at the site of infection or inflammation and in draining versus nondraining lymph node responses. These data support memory responses and trafficking of B and T cells after reinfection or challenge with a highly immunogenic chlamydial antigen.

In summary, there is evidence for acquired immunity in the monkey trachoma models through accelerated clearance after reinfection. Thus, overall, the nonhuman primate studies demonstrated that immunity to conjunctival infections develops with respect to decreasing the chlamydial load, but there was clearly a disconnect between the protective response and the pathologic response, which continued to be strong whenever the animals were given a viable challenge. The reasons for this difference remain to be determined.

SEROTYPE OR GENOTYPE SPECIFICITY OF IMMUNE RESPONSES

As is well known, serovar specificity resides with epitopes of the MOMP and, even with the advent of molecular techniques, has remarkably held to the original serovar designations described by Wang and Grayston [60]. Because the serovar epitopes are very immunogenic, it is clearly important to know whether the protective immune response is dependent on re-

infection with the same serovar or whether it can be generalized to multiple serovars.

Although the concept of serovar specificity was studied to a great extent in the nonhuman primate studies on trachoma vaccines by Wang and Grayston [56], painfully little work had been done in models of chlamydial genital infection. Mice have been inoculated with human serovars and the course of the infections determined, but cross-immunization studies have not been performed [61]. The only study addressing the concept of cross-protection in genital infections was reported by Ramsey et al [62]. In their experiments, mice were infected with either *C. muridarum*, serovar E, or serovar L2 and then cross-challenged with the heterologous strains. Mice given a primary infection with *C. muridarum* demonstrated solid immunity to both human serovars when challenged 56 days later and demonstrated partial immunity to *C. muridarum*. Primary infection with serovar E also resulted in partial immunity to challenge with L2 or *C. muridarum*. Thus, these data suggest that infection with one serovar or species could elicit a protective response to other serovars or species. This is in contrast to findings of some of the earlier nonhuman primate trachoma studies, but it should be remembered that cell-mediated immunity in mice appears to be the most critical response for resolution of infection and immunity to reinfection. Serovar specificity has historically been associated more closely with an antibody response than with a cell-mediated immune response. In fact, in this study, T cells from mice infected with either species or serovar recognized the others in a T cell proliferation assay, indicating that they are recognizing species and/or serovar epitopes.

Although more studies need to be performed, the data thus far suggest that serovar specificity may reside with epitopes recognized by antibodies, whereas the protective T cell response is less sensitive to serovar-specific epitopes. This seems logical, because the surface-exposed epitopes on MOMP appear to determine serovar specificity and whether antibody to MOMP can be effective in a protective response. Batteiger et al [63] observed that immunization of guinea pigs with purified MOMP was effective only if the MOMP was purified using nonreducing methods. In contrast, MOMP obtained using reducing methods elicited a strong antibody response but not a protective response. An important question is whether a vaccine that induces only a T cell response will be sufficient for protection or whether an antibody response is essential for reducing the number of organisms until a T cell response can be mobilized. If an antibody response is desirable, use of a vaccine candidate expressing multiple serovar-specific epitopes or, if possible, one that cross-reacts with many or all epitopes should be considered, but the epitopes should be surface-exposed on the elementary body.

EFFECT OF GENETIC OR PHENOTYPIC DIFFERENCES ON DEVELOPMENT OF IMMUNITY

An important consideration in evaluating the nature of the protective immune response is whether host genetics play a critical role in the development of immunity. Although numerous studies have described the effect of mouse strain differences on the course of chlamydial infection, none have addressed the effect of genetics on resistance to reinfection. Although several studies showed that the course of chlamydial genital infection varies greatly depending on the genetic strain of mouse, all animals ultimately developed a protective immune response and resolved their infections [15, 64, 65]. Indeed, there may be significant quantitative and qualitative differences in the immune response resulting from the primary infection, but nevertheless, the infections resolve. Recently, 2 studies [66, 67] delineated host susceptibility (ie, leading to more severe infection) to specific genetic loci in mice. However, the response to reinfection in various mouse strains with a susceptible phenotype has not been investigated. On the basis of the mouse studies to date, it is probably safe to assume that host genetics and/or virulence of the organism is driving susceptibility to more severe disease by alteration of the host response. Resistance to reinfection does not appear to be affected to any great extent by the genetics of the host, but again, it should be emphasized that this question has not been satisfactorily addressed experimentally.

EFFECT OF ANTIBIOTIC THERAPY ON IMMUNITY

A major factor in an individual immunity to reinfection is whether there has been any previous antibiotic intervention. Because immunity appears to develop somewhat more slowly in humans [68], it is possible that individuals are treated before the development of a protective immune response. Thus, early treatment eliminates the organism and removes the stimulus for the immune response, so that immunity cannot develop. This concept of "arrested immunity" has been put forth by Brunham and Rekart [69] as an explanation for the increased incidence of reported chlamydial genital infection despite established control programs. That this is indeed a viable scenario is supported by animal model data in both mice and guinea pigs. When mice were treated with doxycycline at varying intervals after infection, it was observed that, when treatment was initiated at the time of infection or 3 days later, the mice were completely susceptible to subsequent challenge infection [19]. If treatment was initiated 7 or 10 days after infection, mice demonstrated some immunity but still not at the same level as that of untreated mice. Varying immune parameters, including serum IgG level, genital IgA level, and T cell IFN- γ response,

were correspondingly lower when treatment was initiated early in the infection course. A similar phenomenon was observed in guinea pigs infected genitally with *C. caviae* when tetracycline treatment was initiated early during the infection (N. Schmucker and R.G.R., unpublished data). If therapy was initiated at 3 or 7 days after infection, the guinea pigs were completely susceptible to challenge infection. How this information might be used clinically is problematic, treatment cannot be withheld to increase the potential to develop protective immunity. Nevertheless, the animal data support the arrested immunity hypothesis. Of interest, it should be noted that, although the data from Brunham and Rekart [69] suggest that the development of immunity may be prevented by early antibiotic treatment in the population, they noted that reported case rates of ectopic pregnancy and tubal infertility have decreased [70]; thus, antibiotic therapy appears to be accomplishing the major goal of preventing upper genital tract disease.

SUMMARY

There is clearly a substantial amount of data in animal models to indicate that immunity to chlamydial infection develops after primary infection. Although complete immunity is relatively short, partial immunity can persist for long periods. Complete immunity appears to be dependent on the presence of CD4⁺ Th1 T cells in the genital tract and antibody in the local genital secretions; however, partial immunity appears to be dependent on the presence of IgG in genital secretions, followed by the rapid recruitment of CD4⁺ T cells to the genital tract after reinfection.

Although we know a great deal about the protective immune response, there are still many areas that require further work. In only one genital model study, animals were given >1 challenge infection, and whether the animals demonstrated complete or partial immunity in that study was determined by the time between challenge infections. However, real-life situations for the genital tract have not been modeled. For instance, the effects on immunity of multiple inoculations over several days or of weekly inoculations are unknown. Of interest, studies in the nonhuman primate model for trachoma demonstrated that, with multiple weekly infections, detection of the organism proved to be more difficult with each additional challenge, suggesting the development of protective immunity. If the trachoma studies are a model, an increasingly enhanced immunity over time might be suspected, but ocular infections are clearly different from genital infections. Furthermore, what the effect of challenge with different doses will be and the effect of repeated challenges with varied serovars are unknown. There have been many studies to determine the infectious dose for a primary infection but none to determine the infectious dose for reinfection. In fact, what the actual inoculum is from male to female or female to male is not well understood. The only

animal study of sexual transmission has been in the guinea pig model, in which it was determined that a male at the peak level of infection transmitted $\sim 1 \times 10^2$ – 1×10^3 inclusion-forming units to the female [26]. Whether this is realistic for humans is unknown.

In addition, very little is known about immunity in the male. In the only study of challenge infection in male guinea pigs, the male animals appeared to demonstrate complete immunity for a longer period than did female animals [13]. Of note, when females are infected by sexual transmission from the males, the length of the resulting infection is significantly shorter than that of infection initiated artificially, suggesting some effect of immune mediators in semen [26]. Reinfection through sexual transmission has not been evaluated to determine whether there is also an effect in this situation. However, female guinea pigs were immunized with inactivated *C. caviae* elementary bodies and then challenged sexually and compared with immunized animals challenged artificially with a comparable dose. Immunity to reinfection was observed in both groups; however, similar to the case with primary infection, the duration of the challenge infections in immunized animals was significantly shorter if they were challenged through sexual transmission, again suggesting some effect of semen on the course of infection. Thus, it is clear that there is a great deal to learn about the host response in actual sexual transmission, which leads to contemplation of how meaningful and realistic all of the animal studies on protective immunity really are and how much we can extrapolate from their findings to the human situation.

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