Ocular Pathologic Response Elicited by *Chlamydia* Organisms and the Predictive Value of Quantitative Modeling

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**Background.** *Chlamydia* organisms are a significant cause of ocular and genital tract disease worldwide. Acute inflammatory responses are largely responsible for pathologic changes.

**Methods.** Guinea pigs were inoculated in the conjunctiva with various infectious doses of *Chlamydia caviae*. We developed a predictive model and thresholds of the ocular pathologic response, on the basis of measurements of the pathologic response and chlamydial inclusion-forming unit (ifu) loads, using statistical and mathematical techniques. We validated the predictions by modifying the pathologic response with the use of a lytic chlamydiaphage.

**Results.** If the area under the inclusion-forming unit curve reaches \( \sim 4 \times 10^5 \text{ifu-days} \), then it is likely that an ocular pathologic response will develop and that a serious pathologic finding can develop quickly. The earlier that a pathologic response arises, the longer it will remain. A 2-log_{10} reduction in the peak inclusion-forming unit load reduces the chance of any pathologic finding emerging from 81% to 32%, and it reduces the chance of a serious pathologic finding emerging from 33% to 2%. A reduction in the peak chlamydial load also substantially reduces the duration of the pathologic response.

**Conclusions.** Our predictive model can be used to evaluate the likely effect of interventions that modify the course of chlamydial infection. It suggests that, to be effective in preventing or mitigating pathologic responses, an intervention is required to change the chlamydial time course before the peak inclusion-forming unit load is reached.

*Chlamydia trachomatis* is a significant cause of disease and morbidity worldwide. It is the etiologic agent of trachoma, which is the leading cause of preventable blindness, and the most prevalent bacterial agent associated with genital tract infection in men and women, often leading to female infertility. Infections occurring at both anatomical sites (the eyes and the genital tract) elicit an acute inflammatory response that is primarily responsible for pathologic changes. An adaptive immune response resolves the infection but has also been implicated as contributing to the pathologic response. Although both diseases can be readily treated with antibiotics, antimicrobial treatment of trachoma is logistically difficult because of the widespread nature of the disease in underdeveloped settings. With regard to genital infection in women, the infection is often asymptomatic and progresses to the Fallopian tubes before it is diagnosed; therefore, treatment may be provided too late to prevent salpingitis and tubal obstruction.

It is well recognized that the best option for the prevention of both blindness and infertility caused by *C. trachomatis* is the development of an effective vaccine. However, one of the problems in determining the effectiveness of a vaccine in preventing severe pathologic outcomes, particularly with respect to upper genital tract disease in women, is that, in a clinical trial, it would be impractical to wait sufficiently long to discover whether pelvic inflammatory disease and infertility develop. One alternative is to evaluate other potential markers as predictors of disease of the upper genital tract, such as the...
appearance of particular antigens or antibodies with given specificities. Another potential approach is to develop a mathematical model of some aspect of early genital or ocular disease with the intent of establishing a given pathologic or immunologic threshold that accurately predicts subsequent development of a severe outcome.

In the present study, we used the guinea pig model of chlamydial ocular infection to analyze the pathologic response when guinea pigs were challenged with a variety of infectious doses of *Chlamydia caviae* [1]. We used quantitative techniques to develop a predictive model and thresholds for the development of pathologic outcomes, on the basis of the chlamydial infection time course, and we validated the predictions by modifying the pathologic response with the use of a lytic chlamydiaphage (φCPG1) that was specific for *C. caviae* [2]. The advantage of this approach is that *C. caviae* induces an acute inflammatory response in the conjunctiva that can be easily and accurately graded on a daily basis in individual animals and can then be compared with the quantitative evaluation of chlamydiae in vivo by means of analysis of successive swab specimens. Moreover, we have demonstrated that φCPG1 can infect *C. caviae* in conjunctival infection and, via killing of chlamydiae, can reduce the degree of the pathologic response [3]; therefore, it is an ideal means of testing the predictive ability of the model.

**METHODS**

**Experimental animals and conjunctival infection.** Female Hartley strain guinea pigs (body weight, 500–550 g) were obtained from Charles River Laboratories and were housed individually in filter-topped cages in an environmentally controlled room with a light:dark cycle of 12 h:12 h. All animals were fed food and water ad libitum. *C. caviae* was grown in McCoy cells in accordance with standard methodology for the culture of chlamydiae [4]. Swab specimens were collected from the guinea pig conjunctiva, preserved in 2-sucrose phosphate buffer, and then quantified using a standard methodology for the culture of chlamydiae [4].

Animals that initially were naive to chlamydial infection were inoculated in the conjunctiva of both eyes with the agent of *C. caviae*. This was achieved by depositing 25 μL of inoculum on the eye and then lifting the conjunctiva to allow the fluid to contact the inner conjunctival surface. A total of 50 animals were infected with doses that ranged from $10^2$ to $10^7$ inclusion-forming units (ifu); Five animals each were given $10^2$ or $10^3$ ifu, and 10 animals each were given $10^4$, $10^5$, $10^6$, or $10^7$ ifu. Every 3 days, conjunctival scrapings were obtained from each animal to measure the levels of chlamydial inclusion-forming units. After infection, both eyes of each animal were monitored daily for the development of gross pathologic findings.

Pathologic changes were assessed as described elsewhere [6, 7]. In brief, the palpebral and the bulbar conjunctiva were evaluated for erythema, edema, and exudation in each animal daily at the same time of day. Each observation was classified into 1 of 5 categories: (1) trace pathologic response, (2) slight erythema or edema of either the palpebral or the bulbar conjunctiva, (3) definite erythema or edema of either the palpebral or the bulbar conjunctiva, (4) definite erythema or edema of both the palpebral and the bulbar conjunctiva, or (5) definite erythema or edema of both the palpebral and the bulbar conjunctiva plus the presence of exudate.

**Chlamydiaphage infection.** All chlamydial stocks containing phage were produced by methods described elsewhere [2]. To obtain chlamydia-free phage, phage-infected *Chlamydia* lysate was mixed 1:1 with chloroform to kill the chlamydiae. The chloroform preparation was immediately centrifuged at 10,000 g at 4°C for 5 min, and the aqueous layer was transferred into a clean tube. This suspension was again centrifuged to remove the remaining chloroform. In groups of animals inoculated with *C. caviae* and phage, $10^6$ ifu of *C. caviae* was combined with chlamydiaphage suspension. The amount of phage in the inocula was quantified by real-time polymerase chain reaction performed using the following primers for the phage viral protein 1 (VP1) capsid protein gene: phage-probe primer AGCCTCTGTACGCCGCATCTCAAC, phage-forward primer AAGTCTTTCACAGAACATGGTGTAA, and phage-reverse primer CTTGTTGACCCACATCTCATCCA.

**Analysis.** We calculated the cumulative area under the inclusion-forming units time course curve (figure 1A) for each animal as a statistic that incorporates both the magnitude and the duration of chlamydial infection. The area under the curve is the number of “inclusion-forming unit–days” and is analogous to the epidemiologic measurement of “pack-years” of exposure used to calculate the incidence of lung cancer among smokers [8], for example. To determine the optimal thresholds for the number of inclusion-forming unit–days required for the development of a pathologic response, we produced well-classified frequency curves [9]. The cumulative number of inclusion-forming unit–days for each animal was also associated with the progressive pathologic response, averaged over both eyes for each animal, to determine associations between infection and pathologic findings.

We also used a theoretical inclusion-forming unit time-course function, with statistical relationships ascertained from the empirical data, to predict the expected pathologic outcome of changes in the chlamydial inclusion-forming unit load. The inclusion-forming unit time course $c(t)$ can be denoted by

$$c(t) = \frac{k c_0 \exp \left( \frac{\ln 2}{t_2} - \frac{\ln 2}{t_{1/2}} \right)}{k - c_0 \exp \left( \frac{\ln 2}{t_{1/2}} \right)}$$

where $t$ is the time after infection, $t_2$ is the doubling time for inclusion-forming units, $t_{1/2}$ is the half-life after peak infection,
and \( c_0 \) and \( k \) are fitting parameters denoting the initial inoculum and the theoretical maximal level, respectively. We used data for all 10 animals infected with an initial dose of \( 10^6 \) ifu and a bootstrapping technique \([10–12]\) around a least-squares optimization to estimate the parameters that best fit the observed data. The bootstrapping procedure involved 1000 simulations, which were executed using Matlab (Mathworks); for each simulation, 10 animals were randomly sampled with replacement. The algorithm calculated the optimal values \((t_d, t_{1/2}, k; c_0 = 1)\) that minimized the following objective function:

\[
z(t_d, t_{1/2}, k) = \sum_{i=\text{selected animals}} \sum_{j=\text{all time points}} [c(t_{i,j}; t_d, t_{1/2}, k; c_0) - y_{i,j}]^2,
\]

where \( y_{i,j} \) is the observed inclusion-forming unit load for animal \( i \) at time \( t_j \). By running 1000 simulations, we obtained a simulation-based empirical probability distribution for the function parameters. This resulted in estimation of an inclusion-forming unit doubling time of 3.75 h (95% confidence interval [CI], 3.60–3.93 h), a postpeak half-life of 35.19 h (95% CI, 28.63–44.02 h), and a threshold parameter \( k \) of 5.40 (95% CI, \( 1.90 \times 10^6–11.09 \times 10^6 \) ifu). The optimal parameters from the 1000 simulations were used to generate 1000 likely inclusion-forming unit time courses. These time courses were associated with a mean peak inclusion-forming unit load of 6.74 \( \times 10^5 \) ifu (95% CI, 4.13 \( \times 10^5–9.56 \times 10^5 \) ifu).

We used our simulated inclusion-forming unit time courses, together with results from linear regression analysis of the asso-

Figure 1. Course of chlamydial conjunctival infection (A) and resultant pathologic findings (B) after inoculation with varying doses of *Chlamydia caviae*. Mean inclusion-forming unit loads (A) or pathologic scores (B) for both eyes of each of 5 animals in each dosing group are shown. The pathologic scores were classified as follows: 0.5 denoted a trace pathologic response; 1, slight erythema or edema of either the palpebral or the bulbar conjunctiva; 2, definite erythema or edema of either the palpebral or the bulbar conjunctiva; 3, definite erythema or edema of both the palpebral and the bulbar conjunctiva; and 4, definite erythema or edema of both the palpebral and the bulbar conjunctiva plus the presence of exudate. C, Box plots of the mean duration (expressed as the number of days) of each pathologic finding.

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cation between the observed duration of each pathologic finding and the peak inclusion-forming unit load observed for each animal, to predict the likely pathologic outcome of changes to the inclusion-forming unit time course. For comparative purposes, we used the peak inclusion-forming unit values from our simulated time course over the same sample days, as measured in the animal experiments (i.e., every 3 days).

RESULTS

There was clearly an effect of the C. caviae dose on the kinetics of chlamydial infection and the resulting gross pathologic finding (figure 1). The higher doses resulted in more-accelerated infection that reached greater peak inclusion-forming unit loads, compared with the lower doses (P = .001, by the Kruskal-Wallis test). Similarly, the higher doses elicited more severe pathologic findings (and earlier-occurring pathologic findings) than did the lower doses (P = .0113, by the Kruskal-Wallis test).

The observed mean changes in ocular pathologic findings over time are presented in figure 1, as are the associated mean time course of infection and the distribution of the number of days with each pathologic classification. Similar trends in inclusion-forming unit time courses were observed in most infected animals. There were some differences in the duration and degree of ocular pathologic findings between animals, but the development of serious ocular pathologic responses (erythema or edema of both the palpebral and the bulbar conjunctiva and the presence of exudate) was common (arising in 31 of 50 animals). Serious pathologic responses lasted for a mean of 1.5 days, but the presence of any pathologic response lasted for a mean of 7 days (figure 1C).

To estimate a threshold level of infection for the development of pathologic findings, we constructed well-classified frequency curves that showed the percentage of animals that were correctly classified versus the potential thresholds of the cumulative number of inclusion-forming unit–days (figure 2A). A maximum threshold for the emergence of a pathologic finding was evident at ~4 × 10^5 ifu-days (figure 2A). This finding suggests that, if the area under the inclusion-forming unit curve reaches ~4 × 10^5 ifu-days, then it is likely that some ocular pathologic finding will develop. However, if the area under the inclusion-forming unit curve can be maintained at <4 × 10^5 ifu-days, then a pathologic finding is not likely to develop. The empiricism-based probabilities of type I and type II errors occurring in association with the estimated threshold of 4 × 10^5 ifu-days are 0.15 and 0.28, respectively (figure 2A). To evaluate what incremental change in the number of inclusion-forming unit–days is required to elicit progressive stages of the pathologic finding, we plotted the empirical distribution of the cumulative number of inclusion-forming unit–days when each pathologic stage is reached for all animals (figure 2B). We found that once a trace response arises, pathologic progression occurs quickly (figure 2B). Furthermore, there is a strong negative association between the total number of days with each pathologic stage and the day when the pathologic finding first arises (figure 3). Thus, the earlier the pathologic finding first arises, the longer it will remain. This finding suggests that it is important to interrupt the natural history of infection early after infection. It is anticipated that, to be effective, an intervention is required to change the chlamydial time course before the peak inclusion-forming unit load is reached; not surprisingly, the peak inclusion-forming unit load is highly correlated with the total number of inclusion-forming unit–days (r = 0.976; P < .001), initial inoculum (r = 0.363; P = .001), and the number of days with a pathologic finding (r = 0.542; P < .001).

In table 1, we present summaries from our simulated inclusion-forming unit time courses where the peak inclusion-forming unit load has been reduced by decreasing the growth rate. For each time course, we calculated the total area under the curve and determined the proportion of animals that reached this value and developed each pathologic finding; this is an empirical-based probability of a pathologic finding arising. We also present the expected number of days of duration of each pathologic finding if such a finding does emerge (table 1). We find that even small reductions in the peak inclusion-forming unit load can influence the chance of a pathologic response developing and can decrease its duration. For example, decreasing the peak inclusion-forming unit level by 50% is estimated to reduce the chance of any pathologic finding arising from 81% to 72% and reduce the chance of serious pathologic finding (presence of exudate) arising from 33% to 28%; the expected duration of any pathologic finding decreases from 9.24 days to 8.55 days. However, large changes in pathologic outcomes are only expected in association with relatively large reductions in the peak inclusion-forming unit load. A 2-log_{10} (99%) reduction in the peak inclusion-forming unit load reduces the chance of emergence of any pathologic finding or serious pathologic finding to 32% and 2%, respectively. A 3-log_{10} (99.9%) reduction in the peak load reduces to 22% the chance of any pathologic outcome emerging, and no animals were seen with exudate at this level of infection; here, the expected duration of the pathologic response decreased by almost 4-fold, from 9.24 days to 2.35 days. To reduce the area under the infection curve to less than our estimated threshold of ~4 × 10^5 ifu-days, the peak inclusion-forming unit load must be reduced by at least 88% (~1 log_{10}).

To validate our predictions, we analyzed data from guinea pigs with C. caviae ocular infection that had chlamydaphage added to the inoculum. Infection with phage has been found to modify the kinetics of chlamydial infection in vivo with respect to inclusion-forming unit load and pathologic findings [3]. We made model predictions and comparisons for 2 groups of animals infected in both eyes. Group 1 included 5 animals inoculated with 10^6 chlamydial ifu and 0.41 μg/mL VP1 DNA–equivalent phage, and group 2 included animals inoculated with 10^6
ifu of *C. caviae* and 4.14 μg/mL VP1 DNA–equivalent phage. Similar to data from normal infection, infection curves were fitted through the phage-infection data from each group of animals by performing 1000 bootstrap simulations. This resulted in mean peak inclusion-forming unit loads of $3.75 \times 10^5$ ifu and $1.52 \times 10^5$ ifu for group 1 and group 2, respectively (i.e., the phage-induced interventions resulted in decreases in the peak chlamydial load of ~50% and ~80%, respectively, compared with those associated with the normal course of infection). The animals inoculated with chlamydiaphage also had slower rates of chlamydial growth and experienced delays in the time until the peak load was reached. In table 1, we present the predicted probability of pathologic responses emerging and the expected duration associated with these infection courses (50% and 80% reductions in peak inclusion-forming unit loads). We compare these predicted durations of pathologic findings with the actual number of days that each pathologic stage was observed in the eyes of animals from these groups (figure 4). We note that our predictions are in excellent agreement with the observations; therefore, the mathematical model is able to accurately predict
DISCUSSION

We used data from a standard dose-response experiment coupled with statistical and mathematical techniques to develop a predictive model that establishes thresholds for the development of pathologic outcomes on the basis of the time course of chlamydial infection. The model produced several important observations. First, the finding that the earlier the pathologic finding first arises, the longer it will persist strongly suggests that if one is to interrupt the natural history of infection, the intervention must occur either before infection (e.g., via prophylactic vaccine) or soon after the onset of the infection (e.g., via antimicrobial treatment or therapeutic vaccine). Second, even small reductions in the peak of infection can influence the chance of a pathologic response developing and decrease the duration of the response. Third, major reductions in the pathologic response are expected only if there is a relatively large influence on the peak infection level. Although these observations may seem to be intuitive, the value of the present study is that we have defined and characterized a mathematical model that can be used, at least in the context of the guinea pig ocular model, to accurately predict an outcome of disease on the basis of the single parameter of the chlamydial inclusion-forming unit load. This prediction will be consistent regardless of the mode of intervention.

Not only were experimental data used to generate our model, but we were able to validate the model with an actual intervention by use of an exogenous factor, demonstrating that the model could be used to accurately predict the effect of the intervention. Quantitative models have been used in conjunction with available demographic or disease-associated data to make relatively accurate predictions of clinical outcomes in humans for various diseases [13–20]. However, it is rare for predictive studies associating within-host infection kinetic data with disease progression to have data available on interruptions of infection to test the predictive power of the model [21–24], and this has not previously been done for ocular pathologic findings resulting from chlamydial infection. Inoculation of guinea pig conjunctiva with \( C. caviae \) and its phage (\( \phi \)CPG1) results in a delay in reaching peak levels of infection and a reduced level of pathologic findings in the conjunctiva. When the early data on inclusion-forming unit loads were input into our model, the model was able to accurately predict the extent of the resultant disease.

The observations are dose dependent, with more serious and longer-lasting pathologic responses emerging in association with higher inclusion-forming unit loads (figure 1). It is typical for pathologic findings plus exudate to emerge if the initial inocula are at least \( 10^4 \) ifu (figure 1). For lower inocula, there is a critical balance in determining whether a pathologic response will emerge, and greater inocula are important for determining the time until a pathologic finding first emerges, as well as its duration. Interestingly, waning of the pathologic response tends to commence from approximately day 11 on average, regardless of the preceding time course (figure 1). This is likely to coincide with the pathologic response with a given intervention that modifies the course of chlamydial infection.

![Figure 3](image)

**Figure 3.** The duration of the pathologic response, expressed as the number of days with the pathologic finding, versus the day after infection when the pathologic finding was first observed, for the trace pathologic response \( (A) \), definite erythema or edema of the palpebral or the bulbar conjunctiva \( (B) \), and definite erythema or edema of both the palpebral and the bulbar conjunctiva plus the presence of exudate \( (C) \).
Table 1. The empiricism-based probability of a pathologic finding arising and the predicted number of days with each pathologic finding if it does arise (with the 95% confidence interval [CI]), according to reductions in the peak inclusion-forming unit load.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Normal time course</th>
<th>50%</th>
<th>80%</th>
<th>90%</th>
<th>99%</th>
<th>99.9%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak inclusion-forming unit load</td>
<td>$10^{6.87}$</td>
<td>$10^{6.57}$</td>
<td>$10^{6.20}$</td>
<td>$10^{6.87}$</td>
<td>$10^{6.87}$</td>
<td>$10^{6.87}$</td>
</tr>
<tr>
<td>Estimated total inclusion-forming unit–days, no.</td>
<td>$1.65 \times 10^6$</td>
<td>$1.25 \times 10^6$</td>
<td>$7.03 \times 10^5$</td>
<td>$3.01 \times 10^5$</td>
<td>$3.87 \times 10^4$</td>
<td>$4.59 \times 10^3$</td>
</tr>
<tr>
<td>Trace response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability, %</td>
<td>81</td>
<td>72</td>
<td>60</td>
<td>49</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>Duration, no. of days (95% CI)</td>
<td>9.24 (8.36–10.11)</td>
<td>8.55 (7.86–9.24)</td>
<td>7.70 (7.13–8.27)</td>
<td>6.94 (6.33–7.55)</td>
<td>4.65 (3.39–5.90)</td>
<td>2.35 (0.26–4.44)</td>
</tr>
<tr>
<td>Slight erythema, edema, or bulbar conjunctiva</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability, %</td>
<td>67</td>
<td>61</td>
<td>45</td>
<td>42</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Duration, no. of days (95% CI)</td>
<td>7.46 (6.66–8.26)</td>
<td>6.94 (6.30–7.57)</td>
<td>6.29 (5.77–6.82)</td>
<td>5.72 (5.16–6.28)</td>
<td>3.99 (2.84–5.13)</td>
<td>2.25 (0.34–4.16)</td>
</tr>
<tr>
<td>Definite erythema or edema</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Of palpebral or bulbar conjunctiva</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability, %</td>
<td>55</td>
<td>39</td>
<td>36</td>
<td>35</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Duration, no. of days (95% CI)</td>
<td>4.87 (4.33–5.40)</td>
<td>4.57 (4.14–4.99)</td>
<td>4.20 (3.85–4.55)</td>
<td>3.87 (3.50–4.24)</td>
<td>2.87 (2.10–3.64)</td>
<td>1.87 (0.60–3.15)</td>
</tr>
<tr>
<td>Of both palpebral and bulbar conjunctiva</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability, %</td>
<td>45</td>
<td>33</td>
<td>24</td>
<td>25</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Duration, no. of days (95% CI)</td>
<td>3.12 (2.70–3.55)</td>
<td>2.89 (2.51–3.27)</td>
<td>2.72 (2.41–3.03)</td>
<td>2.56 (2.23–2.89)</td>
<td>2.10 (1.41–2.78)</td>
<td>1.63 (0.49–2.77)</td>
</tr>
<tr>
<td>Other pathology and presence of exudate</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability, %</td>
<td>33</td>
<td>28</td>
<td>17</td>
<td>12</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Duration, no. of days (95% CI)</td>
<td>1.66 (1.27–2.06)</td>
<td>1.58 (1.27–1.90)</td>
<td>1.48 (1.22–1.74)</td>
<td>1.39 (1.11–1.67)</td>
<td>1.12 (0.55–1.69)</td>
<td>0.84 (0.11 to 1.79)</td>
</tr>
</tbody>
</table>

**NOTE.** The 90%, 99%, and 99.9% reductions correspond to 1-log_{10}, 2-log_{10}, and 3-log_{10} reductions, respectively.
with the activation and onset of the adaptive immune response. Within a few days of this point, no observable pathologic finding remains. This may seem contrary to what occurs clinically in humans in whom inflammation-associated pathologic outcomes can be observed after organisms are no longer detected. However, it should be noted that we recorded gross pathologic changes, and it is likely that pathologic changes would be detected microscopically for a considerable time after gross pathologic changes have subsided. Moreover, unlike in humans, inclusion conjunctivitis in guinea pigs has a much shorter course, with infection completely resolving in ~3 weeks.

The observation that inhibition or diminution of the pathologic response is dependent upon early intervention speaks strongly in favor of the necessity for a vaccine against chlamydia genital and ocular disease. Although early intervention after a chlamydial infection may also reduce the level of the pathologic responses, this is especially difficult for genital infections caused by *C. trachomatis*, because of the asymptomatic nature of most infections and the resultant difficulty of making an early diagnosis. However, it is interesting to note that early intervention does indeed reduce the incidence of upper genital tract disease. In an extensive epidemiologic study, Brunham et al. [25–27] observed

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**Figure 4.** Duration of the pathologic finding associated with *Chlamydia* infection, expressed as the mean number of days, for each stage of the pathologic responses of 5 animals inoculated with $10^6$ inclusion-forming units (ifu) of chlamydiae and 4.14 μg/mL VP1 DNA-equivalent phage (A) and of 5 animals inoculated with $10^6$ ifu of chlamydiae and 0.41 μg/mL VP1 DNA-equivalent phage. Gray markers denote the mean expected duration of the pathologic response, expressed as the mean number of days, based on our predictions in table 1 (for an 80% reduction in the peak inclusion-forming unit load). Black markers denote the actual duration of the pathologic response, expressed as the mean number of days, observed for 2 eyes of infected guinea pigs.
that aggressive case detection and contact tracing, followed by antimicrobial treatment, significantly reduced the incidence of pelvic inflammatory disease, infertility, and ectopic pregnancy, although the incidence of infection continued to increase. A key aspect of their study was that early treatment was able to reduce upper genital tract disease in the treated population, an observation that supports our predictions. Nevertheless, a vaccine would be the most effective means of reducing the initial course of infection and decreasing the likelihood of pathologic findings in the upper genital tract. The ability of a vaccine to reduce the number of organisms and, consequently, the degree of pathologic change after ocular challenge was demonstrated in several studies of the use of the vaccine for ocular infections in guinea pig and nonhuman primate models, as well as in several clinical trials of trachoma vaccines [28].

Our mathematical model was derived from an optimal situation in which the subject was infected only once with a known inoculum. However, this study informs us that, in a situation in which the only major variable is the size of the inoculating dose, it is possible to develop a model that accurately predicts the likelihood of disease developing based on early infection parameters. In “real life,” whether they develop trachoma or sexually transmitted infection, individuals are typically infected multiple times over the course of weeks, months, and years, and pathologic findings develop as a result of cumulative exposures. Moreover, the pathologic response is likely to be caused not only by the acute inflammatory response but also by other factors, including the cell-mediated immune response, immune regulatory mechanisms, and reproductive hormones in women with genital infections. Future mathematical models can extend the current model to accommodate additional variables to more accurately characterize the natural situation. Our simple approach has the potential to guide clinical decision-making by predicting the expected pathologic outcome. For example, if the time from inoculation to the emergence of a trace response (and/or other pathologic response) is known, then associations such as those presented in figure 3 can be used to estimate the duration over which the pathologic response could be expected to remain. By comparing this information with the data in the appropriate column in table 1, the relative chance of a more advanced pathologic outcome emerging can be determined along with its expected duration. This value can be cycled back to figure 3 to estimate when a more serious pathologic outcome could emerge. This information could inform the planning of clinical interventions, particularly if this approach was applied to data on chlamydial disease in humans.

One of the major problems associated with both chlamydial genital tract infections and trachoma has been the inability to predict which individuals are more likely to develop severe disease. This is particularly problematic because of the high incidence of genital asymptomatic infections in women. It would be a tremendous advantage if one could use specific parameters, such as (1) the number of inclusion-forming units and/or the level of ≥1 cytokines or chemokines or (2) the number of a given lymphocyte phenotype, to develop a model in which one could accurately predict pathologic outcomes. In the present study, the guinea pig ocular model was used because of the ease of assessment of the pathologic response; however, the same approach can be applied to the genital tract in either the guinea pig or mouse, although, clearly, more animals would be required to assess the pathologic findings in an ascending infection. Future models will incorporate greater complexity, particularly when applied to the mouse genital tract model, because there will be the potential to incorporate variables that describe the effect of various host immune parameters. These models (1) will assist in the evaluation and analysis of interventions in the natural course of chlamydial infection, (2) describe and predict the course of infection and the regulation of the pathologic response, and (3) may eventually guide the assessment of clinical trial candidates and inform likely clinical outcomes in humans exposed to Chlamydia organisms.

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References


