Trachoma: transmission, infection, and control

Manoj Gambhir, María-Gloria Basáñez, Felicity Turner, Jacob Kumaresan, Nicholas C Grassly

Mass antibiotic treatment and facial cleanliness are central to WHO’s strategy for the elimination of blindness caused by trachoma. Recent studies have highlighted the heterogeneous response of communities to mass treatment and the complex relation between infection with *Chlamydia trachomatis* and clinical disease. It is important to be able to explain these findings to predict and maximise the effect of treatment on active trachoma disease and blindness in the community. Here we review the immunobiology of trachoma and provide a simple conceptual model of disease pathogenesis. We show how incorporating this model into a mathematical framework leads to an explanation of the observed community distribution of infection, bacterial load, and disease with age. The predictions of the model and empirical data show some differences that underscore the importance of individual heterogeneity in response to infection. The implications of disease transmission and pathogenesis for trachoma control programmes are discussed.

Introduction

Trachoma is the most common cause of infectious blindness worldwide. In 1996, WHO established the Alliance for the Global Elimination of Blinding Trachoma by the year 2020 (GET 2020), and recommended endemic countries to implement the SAFE strategy (surgery to correct distorted upper eyelids of patients with advanced disease; antibiotic treatment for the infection; facial cleanliness to reduce transmission; and environmental change to increase access to clean water and improve sanitation). Trachoma has recently been identified as a candidate for integrated control with other so-called neglected diseases. A major component of the SAFE strategy is mass administration of the macrolide antibiotic azithromycin, which has been donated by Pfizer to national trachoma control programmes that are implementing the comprehensive strategy. This has provided the incentive to monitor the distribution of bacterial load in the community before and after treatment, and recent studies have measured the rate of reinfection after mass treatment in various epidemiological settings. These studies have shown the heterogeneous response of communities to mass treatment, with the prevalence of infection in some communities showing a sustained reduction, whereas in other communities prevalence rapidly returns to pretreatment levels. Furthermore, the likely effect of a reduction in the incidence of infection on the occurrence of scarring and blindness in later years of life remains unclear. Understanding the relation between the distribution of infection and disease in the population, the role of heterogeneity in the environment and in behaviour, and the response of trachoma-endemic communities to mass treatment remains a major challenge.

We review trachoma immunobiology and mechanisms of disease pathogenesis, and show how these mechanisms lead to observed distributions of bacterial infection and disease in the population. We begin with a brief overview of trachoma immunology and then provide a simple conceptual model of pathogenesis in infected individuals. We then review data on the population distribution of infection, bacterial load, and clinical disease in trachoma-endemic communities. Inclusion of the simple conceptual model of pathogenesis in a mathematical model of transmission reproduces the observed population distributions of infection, bacterial load, active disease, and blindness by age in a series of illustrative fits. Although the general fit of this model to a set of available data is good, the discrepancies underscore the importance of heterogeneity in the immune response of individuals to repeated infection. The Review ends with a discussion of the implications for the effect of control programmes on blindness caused by trachoma.

Trachoma immunobiology and pathogenesis

Our understanding of human ocular *Chlamydia trachomatis* immunobiology rests heavily on research in animals, notably cynomolgus monkeys, and on longitudinal studies of human trachoma pathogenesis in endemic communities. Inference by analogy with animal models and human studies of the better-understood genital chlamydia infections has also played a central part. However, inference from these different systems must be tentative, and indicates differences in host and pathogen biology. For example, interferon-γ-mediated degradation of tryptophan inhibits growth of ocular but not genital serovars of *C trachomatis*, since the latter are able to use indole as a substrate for tryptophan synthesis. With this caveat in mind, we briefly describe the current paradigm for the protective and pathological immune response to ocular infection, some recent challenges to this paradigm, and the role of repeated infection in progression to scarring and blindness.

Trachoma immunobiology

*C trachomatis* possesses a range of mechanisms to evade the immune response of the host, resulting in an infection that takes weeks to clear and that generates only partly protective immunity. On initial infection, epithelial cells secrete cytokines that trigger inflammation and initiate recruitment of immune cells involved in the clearance of infection. Cellular immunity seems to be crucial for the resolution of infection: CD4 T-cell production of interferon γ has been shown to mediate immune clearance of *Chlamydia muridarum* in mice. A preponderance of T-helper type 1 cell cytokines early in infection tends to polarise the response towards an inflammatory protective

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Acquired immunity from past infections appears to explain the shorter duration of infection observed in adults compared with children in a cohort study with frequent (fortnightly) follow-up visits. Although children are highly exposed and reinfection may cause more frequent detection of infection at follow-up, a reanalysis of these cohort data suggests that incidence is not sufficiently high enough to explain, in isolation, the observed longer duration of infection at younger ages (Grassly et al, unpublished data). The acquired immune response seems to involve both cellular and humoral immune components. The protective humoral immune response is targeted at the major outer membrane protein of C. trachomatis, and raised concentrations of IgA antibodies have been shown to be associated with clearance of active trachoma in human beings.

Acquired immunity is only partly protective against reinfection, and repeated episodes of infection have been reported in cohort studies from trachoma-endemic communities. In mouse models of human genital infection, initial challenge results in rapid replication of bacteria resolving in around 4 weeks and, on re-challenge, around half the animals are completely protected. Furthermore, in mice and human trials, vaccination with whole inactivated bacteria confers only partial protective immunity.

**Disease pathogenesis**

Initial infection with ocular C. trachomatis results in a self-limiting conjunctivitis that typically heals without permanent sequelae. However, this results in a hypersensitive state, such that subsequent infection results in more intense inflammation and faster clearance of bacteria. Candidate antigens for inducing this hypersensitive state include the 60 kDa chlamydial heat shock protein, the major outer membrane protein surface antigen, and lipopolysaccharide from the bacterial cell membrane, although none of these antigens in isolation is sufficient to induce progressive disease. Repeated infection results in chronic inflammation, visually apparent as inflamed lymphoid follicles when the upper eyelid is everted. In primates, repeated inoculation with C. trachomatis results in an inflammatory response that may be sustained for as long as the monkey is inoculated.

After years of reinfection and inflammation, scarring of the conjunctiva occurs, which, as it progresses, can cause the eyelashes to turn inwards and abrade the cornea, leading eventually to corneal opacity and blindness. The importance of reinfection for disease progression is reported in a study of Punjabi Indians living in Canada, who, despite signs of previous infection and trachomatous disease, failed to progress further in the absence of exposure to new bacterial inocula.

WHO has devised a simplified system of clinically grading trachoma according to the severity of disease (panel). Because of the central role of reinfection to trachoma pathogenesis, rates of disease progression will depend on levels of exposure and hence overall prevalence of infection. We reviewed studies of the rate of progression from follicular or intense trachoma to scarring, trichiasis, and corneal opacity in different endemic communities (table 1). All cohort studies reporting rates of progression between disease sequelae were included. The rates given by these studies show the long timescales involved in progression to blinding trachoma and indicate the opportunity for surgical intervention.

Exposure varies across communities, both in terms of age and residential structure, and infection clusters within families residing in the same household. However, some infected hosts are more likely to experience severe disease than others, which may in part be explained by heterogeneity in host genotype. Certain HLA alleles seem to be more frequently associated with severe disease, and certain interleukin-10 promoter genetic sequences have been shown to be correlated with conjunctival scarring. Furthermore, some ethnic groups seem to be at greater risk of severe disease than others, suggesting a possible role for host genetic factors in disease.

**Latent bacteria**

Recently, a subset of the host population has been found to be unable to clear infection, and rather than repeated infection driving disease progression, the persistent release of antigen by uncleared bacteria has been proposed to lead to progressive disease. This
hypothesis is difficult to prove or disprove because its signature—persistent, unchanging bacterial serotypes through the course of disease—is also expected as a consequence of the spatial clustering of transmission. In genital infections, interferon-γ-rich environments can induce bacterial persistence, with the continued presentation of antigens such as the 60 kDa chlamydial heat shock protein by this persistent form.

An alternative mechanism for disease pathogenesis has been proposed in which infected epithelial cells produce pro-inflammatory chemokines, cytokines, and growth factors. In this mechanism, the host-acquired immune response has a secondary role in inflammation and scarring, but the primary source of chronic and proliferative cell damage is the infected epithelial cells. This hypothesis can be tested in future research by observing the response profile to infection, which should differ from a pure immune system response if its origin is largely non-immune.

### Epidemiology of infection and disease

In trachoma-endemic communities, the prevalence of active disease (follicular and intense) generally peaks sharply among children aged 1–5 years, in whom it can be higher than 50%, and drops to substantially lower levels in adults, often around (or less than) one-tenth of its peak level. Most cases of active disease in adults are in women, who are in more frequent contact with infectious children, for whom they are the primary caretakers.

The prevalence of infection in communities has recently been studied by use of PCR techniques, whose sensitivity is greater than Giemsa staining, culture, and direct fluorescent antibody procedures previously used. Surprisingly, these studies have found large numbers of PCR-positive adults and a tendency towards a roughly constant prevalence of infection with age. Furthermore, they have indicated the extent of the discrepancy between infection and disease, with typically about one-third of those with active disease showing no detectable infection, and one-third of those with laboratory evidence of infection being classified as clinically well.

Many of the cases of clinical disease in the absence of infection are explained by the kinetics of disease pathogenesis, in which inflammatory disease may persist long after infection has been cleared. The large number of cases of infection without clinical disease is more puzzling. Reasons for this disparity may include, on the one hand, false positives for infection, either because of cross-contamination during examination or poor test specificity, both of which have been identified as potential problems in some early studies, and, on the other hand, low sensitivity of the WHO simplified grading system. (This simplified system was never devised for detailed studies of the relation between infection and disease, and will grade many cases as clinically normal when there are, for instance, less than five inflamed follicles.) A recent study that used

<table>
<thead>
<tr>
<th>Country</th>
<th>Disease prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Gambia</td>
<td>10%</td>
</tr>
<tr>
<td>Tanzania</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Central Tanzania</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Central Tanzania</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Central Tanzania</td>
<td>Hyperendemic</td>
</tr>
</tbody>
</table>

#### Table 1: Studies reporting progression between trachoma disease sequelae

<table>
<thead>
<tr>
<th>Length of study</th>
<th>Frequency of follow-up</th>
<th>Study population</th>
<th>Disease incidence [% transition (95% CI)]</th>
<th>Country</th>
<th>Disease prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowman et al14</td>
<td>12 years</td>
<td>One at 12 years</td>
<td>639 with TS</td>
<td>The Gambia</td>
<td>10%</td>
</tr>
<tr>
<td>Bowman et al24</td>
<td>1 year</td>
<td>6 months and 12 months</td>
<td>190 with TT, all ages, both sexes</td>
<td>The Gambia</td>
<td>..</td>
</tr>
<tr>
<td>Burton et al32</td>
<td>4 years</td>
<td>One at 4 years</td>
<td>154 with TT in one or both eyes</td>
<td>The Gambia</td>
<td>Baseline active 8%</td>
</tr>
<tr>
<td>Muñoz et al26</td>
<td>5 years, 10 years</td>
<td>One at 5 years and 10 years</td>
<td>6038 women, all ages</td>
<td>Tanzania</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Muñoz et al27</td>
<td>7 years</td>
<td>One at 7 years</td>
<td>523 women with TS from 11 villages, &gt;18 years</td>
<td>Central Tanzania</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Taylor et al28</td>
<td>1 year</td>
<td>Every 3 months</td>
<td>53 children from 9 families, age 0-14 years, both sexes</td>
<td>Tanzania</td>
<td>Hyperendemic; peak TF/TI prevalence in children aged 5 years &gt;90%</td>
</tr>
<tr>
<td>West et al29</td>
<td>7 years</td>
<td>Four exams over 1 year at baseline then once at 7 years</td>
<td>98 children with constant severe trachoma, 96 without age 1-7 years</td>
<td>Central Tanzania</td>
<td>Hyperendemic; 60%</td>
</tr>
</tbody>
</table>

CO=corneal opacity. TF=trachomatous inflammation, follicular. TI=trachomatous inflammation, intense. TS=trachomatous scarring. TT=trachomatous trichiasis. ..=not reported.
quantitative PCR for 16S RNA expression has shown that
many individuals positive by qualitative DNA-based PCR
in fact have low bacterial gene expression.48 Instances
that are positive according to DNA-based PCR may
therefore in some cases represent continuous passive
inoculation of the eye with bacteria in the environment,
rather than actively replicating infections.

Several studies have used quantitative DNA-based
PCR to examine the distribution of bacterial load in the
community. These studies reveal substantial
heterogeneity, with the heaviest bacterial loads
concentrated among young children (table 2). In
extreme cases, nearly 50% of all the bacteria in the
sample—the community bacterial load—may be
harboured by children aged under 1 year.30 Adult loads
are generally lower than those of children, presumably
because of effective acquired immunity, although there
is often a small proportion of adults with high loads.45
Again, interpretation of these results should be made
with some caution in view of the potential for cross-
contamination of samples, although this may be
unimportant in these more recent studies, in which
more care has been taken in the handling of samples.

The skewing of infection load towards children is
more marked in communities in which active disease is
hyperendemic (active disease prevalence in children
more than 50%).50 In lower prevalence communities, a
more even distribution of bacterial load is found, with
fewer of the high-load infections occurring in young
children.30 This is consistent with the lower cumulative
exposure to infection experienced by adults in these
communities and the correspondingly lower levels of
acquired immunity. Similar patterns of infection
prevalence and intensity are observed in parasitic
infections such as helminths and malaria.51 The
intracellular replication of Chlamydia spp results in
production of large numbers of infectious elementary
bodies, and individuals with high loads are likely to be
responsible for most new infections in a population.

National surveys often report specific clinical
diagnoses by age, largely according to the WHO
grading system.42,52–55 These surveys report very low
levels of conjunctival scarring, corneal opacity, and
blindness in the youngest age-groups, with the
prevalence of these severe disease sequelae rising
steeply with age. Corneal opacity and blindness
constitute a small fraction of overall disease for older
individuals, whereas scarring often becomes, at older
ages, commensurately prevalent with active disease at
younger ages.56,57 In general, the higher the prevalence
of active disease in the community, the higher the

<table>
<thead>
<tr>
<th>Study</th>
<th>Infection positivity by DNA methods</th>
<th>Infection load distribution</th>
<th>Clinical disease (follicular and intense)</th>
<th>Context and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burton et al4</td>
<td>7% of all ages by qualitative PCR</td>
<td>Mean load in each age-group is fairly constant, but most of load is in &lt;20 year-olds</td>
<td>8% active disease</td>
<td>Upper Saloum district, The Gambia; 14 villages, 1319 individuals examined at baseline.</td>
</tr>
<tr>
<td>Melese et al4</td>
<td>56.3% of those aged 1–5 years by PCR</td>
<td>...</td>
<td>...</td>
<td>Gurage zone, Ethiopia; 1322 children</td>
</tr>
<tr>
<td>Solomon et al30</td>
<td>9.5% of all ages by PCR</td>
<td>Youngest age-groups have highest loads, several with four orders of magnitude greater than older ages</td>
<td>20.4% active disease</td>
<td>Rombo district, Tanzania; one village, 956 individuals tested at baseline.</td>
</tr>
<tr>
<td>West et al4</td>
<td>57% of all ages by PCR</td>
<td>Skewed towards young ages; 71% of those with above median infection load are &lt;15 years</td>
<td>38% active disease (77% prevalence in &lt;8 years)</td>
<td>Kongwa, Tanzania; 873 individuals examined.</td>
</tr>
</tbody>
</table>

---not reported.

Table 2: Studies reporting clinical disease levels and PCR measurements for infection.

Figure 1: A simplified compartmental diagram of the susceptible–infected model of infection
Each susceptible and infected compartment is connected to the compartment above so that the population passes up a ladder of infection (see webappendix for details). i = number of previous infections, =infected stage i. S = susceptible stage i.
(B) Bacterial load in female patients measured by quantitative PCR. (C) Prevalence of active disease for female patients measured as follicular and follicular ± intense. (D) Model-generated prevalence of disease sequelae. (C, D) Error bars represent exact 95% CIs, assuming a random sample of population infection and disease prevalence, respectively. (D) The predicted age profiles of active disease and (E) severe disease sequelae (scarring [purple line] and more severe sequelae [green line]) are also shown; their method of calculation is outlined in the webappendix. The model outputs represent the best fit to the published data (purple squares). Reducing the transmission parameter in the model produces patterns of infection load, active disease, and/or more severe disease sequelae, which is as expected if repeated infection is required for disease progression. 30-40

Mathematical model of trachoma

The central importance of previous exposure to an individual’s biological and clinical response to reinfection can be captured in a simple mathematical model. This model corresponds with a simple susceptible–infected model of infection, 12 but in which each compartment is indexed by the number of previous infections (figure 1). As individuals progress up this ladder of infection, an acquired immune response is assumed to result in more rapid clearance of infection, reduced bacterial load, and more severe disease, in agreement with our understanding of chlamydia immunobiology (webappendix). The probability of infection transmission from an infected individual by direct contact, fomites, or flies is assumed to be proportional to the bacterial load.

This simple model may be fit to cross-sectional or time-series data by the age distribution of infection, bacterial load, active disease, and/or more severe disease sequelae, thus allowing the estimation of parameters describing the relation between rates of clearance of infection or bacterial load and the number of previous infections. We provide a fit of the model to illustrative data collected in a high prevalence trachoma-endemic community (figure 2). Such a close fit of our simple model is reassuring, and suggests that the current paradigm for chlamydia pathogenesis is broadly correct. However, there are some interesting discrepancies. First, the predicted prevalence of active disease in the model drops to zero for older ages, whereas it continues to be reported among a small fraction of older individuals in most trachoma-endemic communities (figure 2). This could be the result of heterogeneity in exposure to infection or the response of individuals to infection, such that a small number are unable to resolve infection and show persistent clinical symptoms. Second, the predicted prevalence of severe disease sequelae in the model rises rather more steeply with age than is commonly observed in endemic settings (figure 2). This might again indicate heterogeneity, missing from the model, in the response of individuals to infection, with some more prone to scarring and progressive disease than others.

Trachoma control

Mass antibiotic administration programmes, using oral azithromycin (at 20 mg/kg bodyweight, up to a 1 g single dose), are being rolled out across Africa, 62 with the aim of eliminating the blinding form of trachoma by 2020. Theoretical and empirical studies are addressing the following key factors: the frequency with which antibiotic treatment needs to be administered to eliminate the infection from a community; the rate at which infection will re-emerge if not eliminated; the main routes and sources for reinfection; and the relative rates of disappearance of infection and disease. Among the theoretical studies, the deterministic mathematical model of Lietman and colleagues 63 investigates how often antibiotic treatment would need to be administered, and at what level of community coverage, to eliminate C. trachomatis infection in a closed population. Because such models do not take into account the stochastic nature of infection in communities, infection always re-emerges at a rate characterised by the initial doubling time (IDT) of the prevalence of infection in the community. The IDT, in turn, can be used to calculate quantities such as the
necessary treatment frequency for consistent (monotonic) prevalence decline. This method has been put to the test in the Gurage zone of Ethiopia, where the required treatment frequency for elimination was estimated at 12 months, provided that 80% of the population is treated, and without immigration of C trachomatis carriers. The IDT is a function of the basic reproduction number, \( R_0 \), which can be estimated from the level of endemic infection observed in communities. In general, the higher the level of endemic infection, the shorter the IDT, the higher the value of \( R_0 \), and the faster the infection will re-emerge. However, the relation between \( R_0 \) and endemic prevalence is complicated by heterogeneity in exposure and the ability to clear infection. Furthermore, the assumption of endemic equilibrium is rarely met. Therefore, in addition to indicating the average behaviour of the host–pathogen system, models of trachoma epidemiology and control will need to capture individual (or household) variability in transmission variables for these models to become truly useful at informing control policy.

Among the empirical studies, we summarise salient findings of longitudinal investigations of trachoma control after a single dose of azithromycin in endemic communities (ie, without re-treatment) in The Gambia, and in Tanzania. Burton and colleagues found that most of the antibiotic-treated villages (oral azithromycin and topical tetracycline ointment, with a therapeutic load over all measurements taken in the village) decreased infection load (measured as the geometric mean bacterial infection load 2 months after treatment, then incident infection in 12 months after treatment, but that travelling outside the village did not increase the risk by 18 months after treatment. Risk-factor analysis revealed that individuals with high infection loads within households therefore seem to be of primary concern to the success of community-based treatment. This study may also indicate that those with a more damaging response, a state of hypersensitivity to one or more bacterial antigens is attained wherein a reintroduction of antigen leads to an immune hyperreaction, rapid clearance of bacteria, and disappearance of active disease, but also severe tissue damage in the wake of this clearance. The reasons for this predisposition to scarring sequelae are currently thought to be linked with immune responses to re-infection that are more damaging to tissue than the responses of those who experience less trachomatous scarring after the same level of exposure. For those with a more damaging response, a state of hypersensitivity to one or more bacterial antigens is attained wherein a reintroduction of antigen leads to an immune hyperreaction, rapid clearance of bacteria, and disappearance of active disease, but also severe tissue damage in the wake of this clearance. This hypersensitive state does not require continued chlamydial infection, and occasional exposure to antigen might be sufficient to lead to the prolonged inflammation that results in scarring.

Ocular infection with C trachomatis precedes subsequent disease and disease often persists beyond bacterial clearance; these infection and disease kinetics explain the many observations in which one of either disease or infection is observed without the other. Clinical examination is currently the only cost-effective method for determining infection prevalence in a community. However, at the individual level clinical diagnosis will miss many cases of infection. Rapid point-of-care assays for the presence of infection address this problem, and may be useful in the future, depending on feasibility and affordability in field situations.

Recent efforts to interrupt transmission of ocular C trachomatis by use of azithromycin have resulted in

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Conclusions

Trachoma disease progression is intimately linked with the immune response provoked by initial and subsequent ocular infections by C trachomatis. Exposure to infection tends to be highly heterogeneous within communities, and additionally, population subgroups may exist who are genetically predisposed to acquire the infection in the first place, and then to develop severe scarring sequelae on continued exposure to infection. The reasons for this predisposition to scarring sequelae are currently thought to be linked with immune responses to re-infection that are more damaging to tissue than the responses of those who experience less trachomatous scarring after the same level of exposure. For those with a more damaging response, a state of hypersensitivity to one or more bacterial antigens is attained wherein a reintroduction of antigen leads to an immune hyperreaction, rapid clearance of bacteria, and disappearance of active disease, but also severe tissue damage in the wake of this clearance. This hypersensitive state does not require continued chlamydial infection, and occasional exposure to antigen might be sufficient to lead to the prolonged inflammation that results in scarring.
new data that document infection and disease prevalence before and after a single-dose treatment. These data show the following: (1) declines in active disease typically lag behind declines in the prevalence of infection after treatment; (2) infection may re-emerge after treatment from low, residual levels or after reintroduction from other areas; (3) high treatment coverage among communities may result in sustained reductions of infection and disease; (4) a population subgroup carrying a high infectious load may respond poorly to treatment and constitute reinfection sources; and (5) the frequency of treatment required for local elimination of infection is dependent on initial endemic levels.

The availability of new data from qualitative and quantitative PCR tools, collected before and during trachoma control programmes, are providing much valuable information on ocular infection with C trachomatis at individual and community levels. Mathematical models of trachoma transmission and control, of a kind able to incorporate these new data, are being developed with the aim of informing control policy for the achievement of the GET 2020 goal.

Our framework has, thus far, focused on feasibly reproducing the progression of individuals through the infection ladder that seems to characterise the epidemiology of trachoma and the progression to ocular disease. Future models will need to be parameterised and fitted to both endemic and post-treatment data to predict the frequency and duration of mass antibiotic administration programmes in settings with different forces of infection, and to identify which members of the community may need to be targeted to reduce reinfection sources that may threaten the overall success of local elimination efforts.

Conflicts of interest
We declare that we have no conflicts of interest.

Acknowledgments
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