Effect of Bacterial Secondary Infection in an Animal Model of Trachoma

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In trachoma the interaction between chronic chlamydial and acute bacterial conjunctivitis has been suggested as important in determining the severity of disease and, therefore, blindness. We investigated the effect of acute conjunctival infection with each of three common human pathogens, Haemophilus influenzae, Haemophilus aegyptius, and Streptococcus pneumoniae, in a cynomolgus monkeys. Although acute conjunctivitis developed, animals with trachoma were not more susceptible to infection than other monkeys, nor did they develop more severe disease as a result of the bacterial conjunctivitis. The failure of bacterial conjunctivitis to exacerbate the experimental trachoma indicates that, in this model at least, chronically maintained chlamydial infection alone is sufficient to produce the changes characteristic of trachoma.

In endemic human trachoma, episodes of bacterial conjunctival infection are generally believed to produce more intense inflammation and trachoma of increased severity, with an increased risk of blindness (2, 4). Although eyes persistently inflammed by chronic chlamydial infection may be more susceptible to chronic bacterial infection, the most florid bacterial conjunctivitis occurs in acute seasonal epidemics which sweep through trachomatous areas (2). Jones et al., in particular, have drawn attention to the interaction of chronic chlamydial conjunctivitis and acute mucopurulent bacterial conjunctivitis (4). It has even been suggested that blindening trachoma does not develop in the absence of bacterial infection (9).

An animal model of trachoma has been developed in cynomolgus monkeys (8) with most of the important clinical and histological features of the human disease. A mixed papillary and follicular reaction develops in the tar sal conjunctiva. This progresses to the production of typical trachomatous scarring. Corneal pannus, however, is not marked. The conjunctival response provides a particularly useful model for the study of human trachoma where blindness usually results from the in-turning of eye lashes (trichiasis) caused by conjunctival scarring. Trichiasis produces corneal opacification and blindness.

Chronic trachoma is produced in this model by the repeated inoculation of the conjunctiva with Chlamydia trachomatis (7) in the absence of appreciable bacterial infection. In this report we present studies undertaken to examine the effect of acute bacterial infection superimposed on chronic chlamydial conjunctivitis in an animal model of trachoma.

MATERIALS AND METHODS

Our standard examination protocol was followed (7, 8). Young, adult, colony-raised cynomolgus monkeys were used. The clinical response of each eye was graded for a number of individual signs using a slit-lamp. The individual signs were then combined to give indices that quantify the clinical response: the follicular index, which characterizes the follicular response, and the inflammatory index, which summarizes the nonspecific signs of inflammation (8). Tears and serum were collected for serological tests, smears were obtained from the superior tarsal plate for cytological study, and conjunctival and nasopharyngeal swabs were taken for chlamydial reisolation cultures in a cycloheximide-McCoy cell tissue culture system and for bacterial reisolation cultures on either blood agar or brain heart infusion agar plates (5). Although both eyes were examined, specimens were taken from the left eye only to eliminate the possibility of artifactitious changes in the right eye.

C. trachomatis Bour, an E serotype, was grown in yolk sacs of embryonated chicken eggs and diluted in phosphate-buffered saline. Each eye was inoculated with 20 μl of a 101.2 50% egg lethal dose suspension of chlamydia.

Three bacterial species were studied. Haemophilus aegyptius (ATCC 1116) was obtained from the American Type Culture Collection, Rockville, Md. Two strains of streptomycin-resistant Haemophilus influenzae type B were examined: strain B Eag, an encapsulated invasive strain, and strain S2, a nonencapsulated strain (5). These strains were provided by R. Moxon, The Johns Hopkins Hospital, Baltimore, Md. The Haemophilus organisms were grown in brain heart infusion broth supplemented with Levitathal base and prepared for inoculation as described by Moxon and Vaughn (5). Each eye was inoculated with 20 μl containing 2×105 organisms. Finally, a pure suspension of Streptococcus pneumoniae type 3 that had been isolated from a patient with conjunctivitis was used. This was provided by P. Charache, The Johns Hopkins Hospital, Baltimore, Md. Again, 20 μl containing 2×107 organisms was inoculated into each eye.

RESULTS

H. aegyptius was inoculated once into both eyes of three groups of monkeys. The first group of five monkeys had experimental "active trachoma" and was receiving weekly ocular inoculations of chlamydia. The second group of four monkeys had "old trachoma"; i.e., they had conjunctival scarring from previous follicular disease, although the weekly inoculation of chlamydia had been stopped and the follicular disease allowed to resolve. The third group was composed of four normal animals. Acute conjunctivitis developed in the normal animals and resolved in 14 days. A
similar transient increase in nonspecific inflammation was seen in both active and old trachoma groups (Fig. 1). Despite this clinical evidence of bacterial infection, the bacteria could not be reisolated from the eye or nasopharynx of any animal. Few 

Haemophilus organisms could be seen on Gram stain, but the number of polymorphonuclear cells increased markedly in most monkeys 2 to 3 days after inoculation and did not resolve until 8 to 14 days after inoculation. 

H. influenzae was inoculated once into both eyes of two monkeys. These organisms could be reisolated from the eyes and the nasopharynx of these animals from the day after inoculation until 4 weeks later. There was a transient increase in the inflammatory index (Fig. 1) but no real change in the follicular response.

Lastly, S. pneumoniae was inoculated into five monkeys with active trachoma and two normal monkeys. All monkeys developed acute conjunctivitis, and organisms were reisolated from the nasopharynx of one of the normal monkeys and three of the monkeys with trachoma from day 1 to day 22 and from the eye of a normal monkey on day 2 and a monkey with trachoma on day 8 (Fig. 2). There was a small transient increase in follicular index in monkeys with trachoma.

There was no change in tear or serum antichlamydial antibody titers in any group. Chlamydial reisolation cultures remained negative in animals with active trachoma despite continuing weekly chlamydial inoculation. No monkey developed new conjunctival scarring during the course of this study.

**DISCUSSION**

Attention has been drawn to the importance of the interaction between chronic chlamydial and acute bacterial infection in areas of endemic trachoma. Jones et al. (4) have called this complex of chlamydial and bacterial infection "communicaible ophthalmia," and the commonest organisms are the 

Haemophilus species, S. pneumoniae, and Moraxella sp. (2, 9). Indeed, some authors have suggested that trachoma in the absence of bacterial conjunctivitis rarely leads to major visual loss (9) and that one of the main mechanisms of action of topical chemotherapy for severe endemic trachoma is the suppression of seasonal epidemics of acute bacterial conjunctivitis (6).
Little attention has been given to examining this question in animal models of trachoma. The feline keratoconjunctivitis agent is a member of *Chlamydia psittaci* and causes a chronic follicular conjunctivitis in cats infected naturally or experimentally. Studying cats, Darougar and co-workers (1) found a synergistic effect between infection with this chlamydial agent and with a streptococcus species isolated from the eye of a cat. However, Howcroft and co-workers (3) did not find any such effect in guinea pigs infected with both the guinea pig inclusion conjunctivitis agent (*C. psittaci*) and *Staphylococcus aureus* isolated from humans. Bacterial infection has not been a feature in the trachoma model of chronic follicular conjunctivitis produced in monkeys with *C. trachomatis*. Although routine bacteriological cultures have not been taken in our previous studies (7, 8), examination of conjunctival scrapings indicates the presence of bacteria in only 5% of these specimens (unpublished data), and ocular pathogens were not recovered from the many cultures taken during the course of the studies presented here.

It is well known that the susceptibility to infection with a given organism varies from species to species. In our present study of experimental bacterial infection in trachomatous eyes, the prolonged ability to recover *H. influenzae* and *S. pneumoniae* clearly indicates that infection was established with these organisms. On the other hand, *H. aegyptius* was not reisolated from any animal, although both control monkeys and those with trachoma did develop acute conjunctivitis and increased polymorphonuclear cells in conjunctival smears, providing clinical evidence for some response to exposure to *H. aegyptius* and presumed infection.

These studies do not demonstrate the marked interaction between the experimental chronic chlamydial and acute bacterial conjunctival infections one would anticipate if concomitant bacterial infection was an essential feature of trachoma. This finding is significant because it indicates that, in our model, the chronically maintained chlamydial infection alone can cause the clinical changes we have observed. However, it does not exclude the possibility of an interaction between bacterial and chlamydial conjunctivitis in human trachoma. Bacterial infection could increase chlamydial transmission by increasing the amount of ocular discharge and thus increase the frequency of reinfection. Alternatively, bacterial infection could simulate repeated reinfection by preventing or delaying the clearance of chlamydial from the conjunctiva. However, our findings suggest that even if bacterial infection could exacerbate the ocular inflammation in endemic trachoma, bacterial infection is not essential for the production of blinding or potentially blinding trachoma, which can be produced by the chlamydial infection itself.

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