

# Immunity to Chlamydial Infections of the Eye

## V. Passive Transfer of Antitrachoma Antibodies to Owl Monkeys

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Previous studies have shown that owl monkeys which have had a trachoma agent infection were subsequently highly resistant to challenge by both homologous and heterologous organisms. In the present study, passive transfer of owl monkey serum containing antitrachoma antibody from immune monkeys did not protect recipient monkeys from infectious challenge with homologous trachoma. Antibody was not detectable in eye secretions of the recipient monkeys until the intensity of infection was waning, suggesting but not proving that local antibody synthesis rather than simple transudation of serum antibody occurs.

The owl monkey (*Aotus trivirgatus*) has been used as an animal model for trachoma agent infection in man (1). The chronicity characteristic of the disease process in man is lacking, but the acute infection is similar and warrants study because of its potential for insight into the human disease. Experimentally induced trachoma in the owl monkey thus produces acute purulent conjunctivitis with numerous inclusions in conjunctival epithelial cells. These symptoms and signs are observed consistently when monkeys are infected with at least 20 mean egg infectious doses (EID<sub>50</sub>) of trachoma agent (1, 6).

In a previous study when owl monkeys were challenged 70 days after a primary trachoma infection, inclusion rates were 25- to 200-fold lower after infectious challenge than after the initial infection (6). The present experiments investigated the nature of this resistance to reinfection by determining whether passive transfer of serum antibody would protect recipient monkeys against challenge by a minimal infective dose of trachoma.

### MATERIALS AND METHODS

**Owl monkeys.** Owl monkeys (*A. trivirgatus*) were obtained from a commercial importer by the New England Regional Primate Research Center. Prior to this study, all monkeys were free from clinical signs of trachoma infection and were negative for serum and eye secretion antibodies to HAR-13, HAR-32, and HAR-36 trachoma antigens (representing all three serotypes of ocular trachoma) as measured by indirect immunofluorescence. In addition, there was no evidence of trachoma inclusions in their conjunctival epithelial cells as judged by immunofluorescent staining (8).

**Trachoma.** Trachoma agent strain TRIC/1/

ET/HAR-13/OT (HAR-13) was used both to infect the serum donor monkeys and to challenge the serum recipient monkeys. The trachoma organism was maintained, cultivated, and titered in chicken embryo yolk sacs as previously described (4, 6).

**Infection of serum donor monkeys.** The two serum donor monkeys (D1 and D2) were each previously exposed to various preparations of HAR-13 over a 3-month period: (i) direct inoculation into each eye with infectious trachoma agent, which resulted in a typical infection as documented by inclusions seen in conjunctival cells by immunofluorescent examination; (ii) intradermal injections of trachoma skin test preparations; and (iii) intramuscular injection of trachoma in Freund complete adjuvant. Five months after this last exposure to these trachoma preparations, serum titers of both monkeys were 1:2,560 to HAR-13 by immunofluorescence. At this time, each donor monkey was inoculated (directly into each eye) with 20  $\mu$ liters of a 1:10 dilution of crude yolk sac containing infectious trachoma organisms. Previous titration had shown this to be a large infectious dose (2,000 EID<sub>50</sub>).

After 14 days, these animals were again given identical doses of the infectious organism. Ten days after this second dose of trachoma agent, the monkeys were killed, and their sera were aseptically collected and stored at 4 C.

With one exception, these monkeys appeared totally resistant to reinfection; conjunctival cells of these monkeys (on days 0, 14, 21, and 24) did not contain any inclusions in over 66,000 cells examined. One monkey (D1), however, had an inclusion rate of 0.4 (4 inclusions in 10,000 cells) on a single day: the day its serum was collected (day 24). These data were interpreted as consistent with strong resistance to infection.

Two control donor monkeys (D3 and D4) without previous exposure to trachoma agent were treated identically except that they received the

same amount of uninfected yolk sac instead of yolk sac-grown trachoma.

**Antibody determinations.** Fourfold dilutions of serum and eye secretions were tested for antibody to HAR-13 slide antigen as previously described (8).

**Serum transfer.** Three recipient test monkeys (R1, R2, and R3) each received intraperitoneally 9.0 ml of serum containing antitrachoma antibodies. The antibody-containing serum from each of the two donor monkeys (D1 and D2) had an immunofluorescence titer of 1:10,240. Two control monkeys (R4 and R5) each received 9.0 ml of antibody-negative serum, and one control monkey (R6) received no serum (Table 1).

**Infectious ocular challenge of serum-recipient monkeys.** Three days after serum transfer, test and control monkeys were challenged by the inoculating into each eye 20 EID<sub>50</sub> of trachoma organisms contained in 20  $\mu$ liters of a 1:1,000 dilution of crude yolk sac. In our experience this is the lowest dose which will insure infection of all monkeys challenged (6).

**Microbiological measurement of infection.** Intensity of infection was quantitated by inclusion rates (inclusions per 1,000 conjunctival epithelial cells). Conjunctival cells were taken from the tarsal plate of the everted upper eyelid with a blunt dental scraper and transferred to a microscope slide. The smear was allowed to air-dry and was then acetone-fixed for 10 min. Slides were stored at -20 C and subsequently stained with

fluorescein-conjugated lymphogranuloma venereum antiserum, as previously detailed (4).

**Eye secretions.** Eye secretions were collected with heat-sterilized Weck-cel sponges (Edward Weck and Co., Long Island City, N.Y.) as previously described (3). Samples from the right and left eyes of a given monkey were pooled by dropping both sponges into 0.4 ml of phosphate-buffered saline (0.15 M sodium phosphate, 0.85% sodium chloride, pH 7.2) for an initial dilution of approximately 1:5.

**General procedures.** Each monkey was sedated with 0.05 ml of phenylelidine hydrochloride (Sernylan, Parke Davis and Company, Detroit, Mich.). The presence and severity of ocular discharge was determined by gross clinical examination. After eversion of the eyelid, the degree of hyperemia was recorded. Eye secretions were taken, and blood was then drawn. When performed, serum transfer and infectious challenge were the last procedures to be done on that particular day.

## RESULTS

**Antibody in recipient monkeys.** The serum and eye secretion antibody titers are shown in Tables 2 and 3, respectively. The three monkeys which received passively transferred serum had levels of circulatory antitrachoma antibody at day zero (3 days after receipt of donor sera, Table 2) which were similar to levels which previously have been correlated with resistance to challenge (3). No antibody was detectable in the eye secretions of these recipient test monkeys after serum transfer until day 28 (Table 3).

**Microbiological evidence of trachoma infection.** The rate of inclusions was calculated on the basis of total inclusions in both eyes (Table 4). All monkeys became infected after the trachoma challenge. An analysis of variance did not indicate any significant differences at the 95% confidence level between the severity of infection in control and antibody recipient groups. The circulating antitrachoma antibody passively transferred to the test group of monkeys did not protect against homologous challenge (Table 4).

## DISCUSSION

Trachoma agent infection in owl monkeys has been shown to confer resistance against ocular challenge (3, 6). In additional experiments in this laboratory (unpublished data), serum antibody titers of between 1:40 and 1:640 (in owl monkeys infected with trachoma) have been correlated consistently with either partial or complete protection to challenge with 200 EID<sub>50</sub> of infectious organism.

To determine the role of circulating antibody in the host's response to ocular trachoma infection, the present study was designed.

TABLE 1. Summary of antibody titers<sup>a</sup> of donor and recipient monkeys in serum transfer studies

Monkey no.	Pre-transfer reciprocal serum titer <sup>b</sup>	Post-transfer reciprocal serum titer <sup>b</sup> (day zero)
Donor		
D1	10,240	
D2	10,240	
D2	10,240	
D3	<10	
D4	<10	
None		
Recipient		
R1	<10	640
R2	<10	160
R3	<10	640
R4	<10	<10
R5	<10	<10
R6 <sup>c</sup>	<10	<10

<sup>a</sup> Titers of donor sera and titers present in sera of recipient monkeys on day of infectious challenge (day zero, 3 days after serum transfer). A titer of <10 is considered negative.

<sup>b</sup> Reciprocal immunofluorescent serum titers to HAR-13 trachoma antigen.

<sup>c</sup> Monkey number R6 did not receive any control serum.

TABLE 2. Reciprocal of serum antibody titers to trachoma virus in sera of recipient monkeys<sup>a</sup>

Monkey no.	Serum antibody titers on days postchallenge <sup>b</sup>													
	-15	-3	-2	-1	0	5	7	10	12	14	17	21	28	35
Test														
R1	<10	<10	640	640	640	640	640	640	640	640	640	640	640	160
R2	<10	<10	160	160	160	160	160	640	160	40	Died			
R3	<10	<10	160	640	640	160	160	160	160	160	160	160	160	160
Control														
R4	<10	<10	<10	<10	<10	<10	<10	<10	40	160	NT <sup>c</sup>	640	160	160
R5	<10	<10	<10	<10	<10	<10	<10	40	10	40	160	160	2,560	2,560
R6	<10	<10	<10	<10	<10	<10	<10	<10	160	640	2,560	2,560	10,240	40,000

<sup>a</sup> Serum antibodies in all test monkeys (R1, R2, and R3) were derived from passively transferred sera. No serum antitrachoma antibody was detected in control monkeys until day 10.

<sup>b</sup> Days with minus signs are times of serum transfer; other days are those of ocular challenge with 20 EID<sub>50</sub> of trachoma organisms.

<sup>c</sup> NT, No test.

TABLE 3. Reciprocal of antibody titers to trachoma virus in eye secretions of recipient monkeys

Monkey no.	Reciprocals of antibody titers on days postchallenge					
	12 <sup>a</sup>	14	17	21	28	35
Test						
R1	<10	<10	<10	<10	40	10
R2	<10	<10	Died			
R3	<10	<10	<10	<10	10	10
Control						
R4	<10	<10	<10	<10	40	40
R5	<10	<10	<10	40	40	40
R6	<10	10	160	160	40	

<sup>a</sup> On days -3, -2, -1, 0, 5, 10, and 12 postchallenge, no eye secretions contained measurable antitrachoma antibody.

The transferred serum described above was derived from monkeys which seemed immune to 2,000 EID<sub>50</sub> of trachoma virus. Serum antibody levels in the three monkeys which received serum containing antitrachoma antibody (R1, R2, and R3) reached titers of 1:640, 1:160, and 1:640, respectively. These monkeys showed no significant immunity to homologous challenge (20 EID<sub>50</sub>) either in terms of lower inclusion rates or in a delay in the onset of infection (Table 4). If even partial protection had occurred, it would be expected that the minimal infective dose of trachoma used as an infectious challenge (20 EID<sub>50</sub>) would not have broken through a small degree of immunity. Thus the failure to protect is

TABLE 4. Inclusion rates of owl monkeys challenged with 20 EID<sub>50</sub> of trachoma<sup>a</sup>

Monkey no.	Inclusion rates on days postchallenge					
	-15	5	7	10	12	14
Test						
R1	0.0	0.5	243.3	118.0	0.0	17.7
R2	0.0	0.0	13.3	114.7	71.5	45.5
R3	0.0	0.0	21.6	11.6	8.7	9.0
Control						
R4	0.0	0.2	25.0	65.6	6.7	3.7
R5	0.0	0.0	0.0	51.9	70.0	9.4
R6	0.0	0.4	29.9	9.2	4.9	2.5

<sup>a</sup> Inclusion rates (inclusions/1,000 conjunctival cells) as detected by the immunofluorescence. Analysis of variance did not show significant differences between test and control groups of monkeys at the 95% confidence level.

not likely due to either (i) insufficient prechallenge antibody levels in recipient monkeys or (ii) too massive a challenge dose of infectious organism.

The fact that passively transferred antitrachoma antibody did not protect against challenge is consistent with the analogous findings in similar studies in the guinea pig by Watson et al. (9). Passively transferred serum antibody in our study neither delayed nor attenuated ocular infection. By contrast, none of the previously infected control animals was susceptible to reinfection although many had lower titers of circulating antibody than did our antibody recipients. Moreover, in recipients of the passively transferred

antibody as in primarily infected control animals, eye secretion antibody appeared and rose only 12 days after passive transfer, and only after 11 days of infection, suggesting but not proving that local synthesis rather than transudation of serum antibody occurs. In conclusion, serum antibody seems relatively unimportant in resistance to ocular guinea pig inclusion conjunctivitis infection. Beyond this, implicit in our data is the suggestion of a need for further evaluation of secretory and cellular immune mechanisms in mediating resistance to this chlamydial infection.

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#### LITERATURE CITED

1. Bell, S. D., and C. E. O. Fraser. 1969. Experimental trachoma in owl monkeys. *Amer. J. Trop. Med. Hyg.* **18**:568-572.
2. Elliff, J. E. 1967. A method for corneal suture removal. *Amer. J. Ophthalmol.* **63**:346-382.
3. McComb, D. E., J. H. Peters, C. E. O. Fraser, E. S. Murray, and R. L. Nichols. 1971. Resistance to trachoma infection in owl monkeys correlated with antibody status at the onset in an experiment to test the response to topical trachoma antigens, p. 396-406. *In* R. L. Nichols (ed.), *Trachoma and related disorders caused by chlamydial agents*. Excerpta Medica, Princeton.
4. Murray, E. S., S. D. Bell, Jr., A. T. Hanna, R. L. Nichols, and J. C. Snyder. 1960. Isolation and identification of strains of elementary bodies from Saudi Arabia and Egypt. *Amer. J. Trop. Med. Hyg.* **9**:116-124.
5. Murray, E. S., and L. T. Charbonnet. 1971. Experimental conjunctival infection of guinea pigs with the guinea pig inclusion conjunctivitis organism, p. 376-396. *In* R. L. Nichols (ed.), *Trachoma and related disorders caused by chlamydial agents*. Excerpta Medica, Princeton.
6. Murray, E. S., C. E. O. Fraser, J. H. Peters, D. E. McComb, and R. L. Nichols. 1971. The owl monkey as an experimental primate model for conjunctival trachoma infection, p. 386-395. *In* R. L. Nichols (ed.), *Trachoma and related disorders caused by chlamydial agents*. Excerpta Medica, Princeton.
7. Nichols, R. L., A. A. Bobb, N. A. Haddad, and D. E. McComb. 1967. Immunofluorescent studies of the microbiologic epidemiology of trachoma in Saudi Arabia. *Amer. J. Ophthalmol.* **63**:1372-1408.
8. Nichols, R. L., and D. E. McComb. 1962. Immunofluorescent studies with trachoma and related antigens. *J. Immunol.* **89**:545-554.
9. Watson, R. R., J. D. Mull, A. B. MacDonald, S. E. Thompson III, and S. E. Bear. 1973. Immunity to chlamydial infections of the eye. II. Studies of passively transferred serum antibody in resistance to infection with guinea pig inclusion conjunctivitis. *Infect. Immunity* **7**:597-599.