INFECTION AND IMMUNITY, Apr. 1973, p. 597-599

Vol. 7, No. 4
Printed in U.S.A.

Immunity to Chlamydial Infections of the Eye

II. Studies of Passively Transferred Serum Antibody in Resistance to Infection with Guinea Pig Inclusion Conjunctivitis

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Received for publication 24 October 1972

Conjunctival infection of guinea pigs by the chlamydial agent of guinea pig inclusion conjunctivitis confers immunity. However, the mechanism of resistance to this intracellular pathogen is not yet defined. In the study reported here, serum immunoglobulin was passively transferred with resultant titers in excess of those known to be associated with immunity. Nonetheless, when the passive transfer recipients were challenged, they acquired infection which was neither delayed nor attenuated. Eye secretion antibody titers appeared and increased only after 11 days of infection in both passive transfer recipients and control groups, suggesting but not proving de novo local synthesis of secretory antibody. This study suggests that cellular or secretory immune mechanisms may predominate in resistance to this infection.

Guinea pig inclusion conjunctivitis (GPIC) is studied as an animal analogue of human trachoma (3-5). Although it lacks the chronicity which characterizes the human disease, the acute infection in the guinea pig warrants study because of its potential for elucidating the primary trachoma infection in man. In the guinea pig, GPIC infection results in an ocular discharge and intracellular chlamydial inclusions in conjunctival cells; both of these signs disappear within about 3 weeks. After this, immunity to subsequent infectious challenge is present for at least 3 months (4, and unpublished data).

The immune mechanisms which confer resistance to chlamydial infection in the guinea pig, as in man, are undefined. This study was conducted to determine the role of circulating antibody in protection against ocular chlamydial infections in the guinea pig.

MATERIALS AND METHODS

Experimental animals: immune serum donors and previously infected controls. Hartley breed guinea pigs were used. These animals came from stock which has been maintained serologically and microbiologically free from GPIC infection for more than 5 years. One group of 59 animals was isolated from the others 2 months prior to the experiment and infected with GPIC as follows. The inoculum used in all instances contained approximately 100 guinea pig mean infective doses of yolk sac-grown GPIC agent (3) diluted 2×10^{-3} in phosphate-glutamate-sucrose buffer, pH 7.2 (1). Each eye received an inoculum of 0.01 ml. Infection resulted in all cases and was documented by observation of inclusions in Giemsa-stained conjunctival cells (5). Fifty of these animals were used as immune serum donors and nine were used as "previously infected immune controls." Two months after their first infection, none of these nine previously infected immune control animals became infected upon a second infectious challenge.

Microbiological evidence of infection. Conjunctival infection was evaluated by microscope examination of Giemsa-stained conjunctival cells from the upper eyelid (3, 5). Both the number of infected cells per 100 conjunctival cells and the number of inclusions per 100 conjunctival cells were used as measures of the intensity of infection.

Antibody determination in eye secretions and serum. Eye secretions were collected from ether-anesthetized animals with the fire-polished end of a disposable 25-μliter pipette (Clay Adams Co., Parsippany, N.J.). Blood was obtained by cardiac puncture. Eye secretions and sera were stored at −70 C. Eye secretions were titered for antibody to GPIC by using a four-layer immunofluorescence test as follows. The slide antigen was a standardized mixture of GPIC organisms in 10% yolk sac, acetone fixed on a microscope slide. Eye secretions were then placed on the dried layer and incubated at 37 C in a moist atmosphere for 1 h. After rinsing and air-drying, rabbit anti-guinea pig colostrum antiserum, reactive with guinea pig immunoglobulins (Ig) IgG, IgA, and secretory IgA, was placed on the layer, incubated, rinsed, and dried. Finally, the fourth layer, consisting of goat anti-rabbit IgG conjugated with fluorescein, was added. Slides were read immediately after

preparation. All sera were titered with an indirect fluorescent-antibody test by using a rabbit anti-guinea pig gamma globulin conjugated with fluorescein isothiocyanate with GPIC slide antigen, as described above.

Isolation of anti-GPIC immunoglobulin fraction. Serum was obtained from the experimental donor animals at the height of their immunity, 35 to 50 days after infection. Serum was obtained from control donor animals 35 to 50 days after inoculation with 0.01 ml of a 2×10^{-3} dilution of normal yolk sac in each eye. All sera were centrifuged at $34,000 \times g$ for 20 min at 4 C, and then delipidized by filtration through glass wool. Immunoglobulins were precipitated and concentrated from the normal and immune serum pools by precipitation from 18% sodium sulfate at 25 C, suspended in 0.1 M borate-buffered saline (pH 8.0), and dialyzed against 0.1 M phosphate-buffered saline (pH 7.2) before use. The immune immunoglobulin contained 26.5 mg of proteins per ml, as measured by the Lowry method (2). The normal (control) immunoglobulins contained 16.5 mg of protein per ml. Each represented a fourfold concentration over the original serum. The immunoglobulin fraction from the immune animals had an anti-GPIC titer of 1:320; the immunoglobulin fraction from the normal animals was negative at a 1:5 dilution.

Immunoglobulins were diluted 1:7 in phosphate-buffered saline and injected intraperitoneally into 2-week-old, 50- to 70-gm guinea pigs. The animals receiving the anti-GPIC immunoglobulin fraction were designated "anti-GPIC Ig recipients." The animals receiving the normal (control) immunoglobulin were designated "control Ig recipients."

Infectious challenge. The infectious inocula were grown in yolk sac prepared and titered as previously described (3, 5). Twenty-four hours after passive transfer (day 0), recipients and normal and immune control animals were inoculated with 10 guinea pig mean infective doses of GPIC in 0.01-ml volume in each eye.

Experimental design. As described above, these experiments began with the infection of 59 animals, which 45 days later were used either as serum donors or as previously infected immune controls. Serum was harvested from 50 of these animals on day −2 and passively transferred to the recipient animals on day −1. Likewise, immunoglobulins from normal animals were passively transferred on day −1 to another group of normal animals as a control for the antibody transfer group. On day 0, an infectious challenge of GPIC organisms was inoculated into the eyes of four groups of animals: (i) the passive transfer recipients of antibody to GPIC, (ii) the control animals which were recipients of nonimmune immunoglobulin from normal nonimmune animals, (iii) the previously infected and immune animals, and (iv) normal controls.

RESULTS

Antibody in recipient guinea pigs. Geometric mean serum titers in the six animals which received immunoglobulin from guinea pigs immune to GPIC were 1:100 on the day of challenge and declined slowly thereafter (Fig. 1). No antibodies were found in eye secretions of this group on day 0, the day of challenge. The six control animals had no detectable antibodies in sera or eye secretions on day 0. Eleven days after challenge, antibody titers began to rise in both sera and eye secretions of control animals and in the eye secretions of the test group (Fig. 1).

Antibody in control guinea pigs. The nine previously infected immune controls all had circulating antibody with titers ranging from 1:7 to 1:112 and eye secretion antibody with titers ranging from 1:16 to 1:64 on day 0. After repeat infectious challenge, antibody levels rose slightly in all control animals so that on day 11, serum antibody titers ranged from 1:28 to 1:224 and eye secretion antibody titers ranged from 1:16 to 1:256. As noted below, however, no infection could be detected.

Intensity of microbial infection. As shown in Fig. 1, there is no statistical difference in the microbiological intensity of infection between anti-GPIC recipients and Ig recipient control groups (unpaired t test, $P > 0.5$). Neither was it possible to distinguish between the groups in the

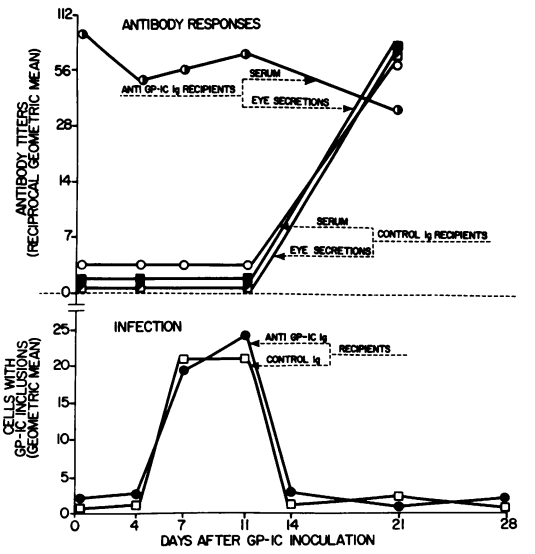

FIG. 1. *Antibody responses and infection in recipients of passively transferred immunoglobulins. Anti-GPIC Ig recipients received antibody to GPIC from immune animals. Control Ig recipients received immunoglobulins from normal animals.*

intensity of infection by the number of polymorphonuclear leukocytes present with the conjunctival cells nor by the number of elementary bodies per infected cell. The immune control animals were totally resistant to infection by both clinical and microscopic criteria. The normal control animals developed a characteristic infection.

DISCUSSION

Conjunctival infection of guinea pigs with the GPIC agent results in immunity (3–5). Both serum antibody and eye secretion antibody develop subsequent to conjunctival infection (E. S. Murray, L. L. Charbonnet, and A. B. MacDonald, J. Immunol., in press); the role of these antibodies, as in the case of human infection with trachoma, has not been defined. In the work reported here, we studied the role of serum antibody by means of passive transfer experiments. Murray and Charbonnet reported that guinea pigs injected intraperitoneally with killed formalinized GPIC agent developed high titers of circulatory antibody but were not resistant to infection (4). In the present studies of passive transfer of serum antibody resulting from live ocular GPIC infection, we added to the work of Murray, and the data recorded in this report demonstrate that serum antibody from animals rendered immune to reinfection by previous conjunctival infection does not convey protection. 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 the present study, 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 local synthesis rather than transudation of serum antibody. In conclusion, serum antibody seems relatively unimportant in resistance to ocular GPIC 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.

ACKNOWLEDGMENTS

This work was supported by the Seeing Eye, Inc., Morristown, N.J., by Public Health Service grants 9-ROI-EY-00812 from the National Eye Institute and AI-00177 (training grant) and 5-TOI-AI-00221 from the National Institute of Allergy and Infectious Diseases, and by a grant from the Arabian American Oil Co., Dhahran, Saudi Arabia.

We are deeply indebted to Edward S. Murray, whose advice was vital to us at every stage of our work. We also gratefully acknowledge the expert technical assistance of Leslie A. Sullivan.

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