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Specific Effect of Estradiol on the Genital Mucosal Antibody Response in Chlamydial Ocular and Genital Infections

ROGER G. RANK* AND ALMEN L. BARRON

Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

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Estradiol treatment of female guinea pigs was found to alter the course of genital, but not ocular, infection with the chlamydial agent of guinea pig inclusion conjunctivitis. Immunoglobulin G (IgG) and IgA responses in genital secretions of genitally infected animals were delayed by estradiol treatment, but neither response in the eye resulting from either ocular or genital infection was affected. However, the appearance of IgG in the genital tract after ocular infection was markedly inhibited in estradiol-treated guinea pigs.

Several studies of women have suggested that chlamydial genital infection may be influenced by reproductive hormones either as a result of the natural menstrual cycle (14) or by administration of oral contraceptives (15). Previous studies in our laboratory have demonstrated that pharmacological or physiological doses of estradiol given parenterally to female guinea pigs have both prolonged genital tract infection with the agent of guinea pig inclusion conjunctivitis (GPIC) and increased the incidence of ascending infection of the uterus and fallopian tubes (9, 12). Guinea pigs treated with estradiol had a marked increase in the number of infected cells in the genital tract early in infection (12), implying some physiological effect of the hormone on the chlamydia-host cell interaction, which has been supported by in vitro experiments (2, 8).

It has also been observed that estradiol treatment delayed the appearance of immunoglobulin G (IgG) and IgA in genital secretions but not serum (9, 10). Whereas previous experiments only measured the effect of estradiol on local development of genital antibodies resulting from genital infection, we felt that it was important to determine whether the delay in the appearance of antibodies was a phenomenon specific to the genital tract or whether it encompassed other mucosal sites as well. To investigate this possibility, we treated groups of female guinea pigs with estradiol and infected one group ocularly and the other genitally. Both ocular and genital secretions were collected from both groups of animals, and the kinetics of the local antibody responses were determined and compared with those of control groups similarly infected.

Female Hartley strain guinea pigs (Charles River Laboratories, Wilmington, Mass.), weighing 450 to 500 g, were inoculated intravaginally with 0.05 ml of a GPIC suspension grown in either McCoy cells or yolk sacs (1). Ocular infection was accomplished by dropping 0.03 ml onto the eye so that the conjunctivae were in contact with the fluid. The course of the infection was monitored by obtaining conjunctival or vaginal scrapings and determining the percentage of cells containing chlamydial inclusions on a Giemsa-stained smear (1).

Serum samples and genital secretions were collected as previously described (11). Ocular secretions were collected with sections (2 by 3 mm) of Weck-Cel sponges (Edward Weck and Co., Inc., Durham, N.C.) by swabbing the conjunctivae with the sponges. Secretions were eluted in phosphate-buffered saline (pH 7.2) containing 0.05% sodium azide (0.2 ml/0.1 g of secretion). For titration purposes, this final suspension was considered undiluted.

Antibody titers were determined by an enzyme-linked immunosorbent assay modified from that described by Levy et al. (7). Microtiter plates were coated overnight with gradient-purified GPIC antigen (3) suspended to 5 μg/ml in 0.5 M carbonate buffer. All incubations were conducted at 37°C. The plates were washed with phosphate-buffered saline containing 0.05% Tween 20 and 5% calf serum and incubated for 1 h with the buffer. Twofold dilutions of either serum or secretions, beginning with a 1:10 dilution, were made in the same buffer containing 2% calf serum (PBS-Tw-2) and incubated for 1 h. To determine IgG titers in serum or secretions, peroxidase-labeled rabbit anti-guinea pig IgG (H and L chain specific; Miles Laboratories, Elkhart, Ind.) diluted in PBS-Tw-2 was added for 1 h. For measurement of IgA in secretions, rabbit anti-guinea pig alpha chain (Miles Laboratories) was added for 1 h, followed by peroxidase-labeled goat anti-rabbit IgG (H and L chain specific; Miles Laboratories). The plates were developed by addition of a solution of 5-aminosalicylic acid (4) with 0.005% H_2O_2 and allowed to develop overnight. The titer was considered to be the last well having an optical density (492 nm) of at least 0.1 for IgG and 0.270 for IgA.

In the first experiment, two groups of four animals each were injected subcutaneously with 0.1 ml of sesame oil containing 1,000 μg of β-estradiol-3-benzoate (Sigma Chemical Co., St. Louis, Mo.) daily beginning 6 days before infection and continuing for the course of the experiment. Two control groups were injected similarly with sesame oil alone. On day zero, one group each of estradiol-treated and sesame oil-treated guinea pigs was inoculated ocularly with McCoy cell-grown GPIC containing 5.2×10^5 inclusion-forming units, while the remaining two groups were inoculated intravaginally with 8.6×10^5 inclusion-forming units. Since 1,000 μg of estradiol results in pharmacological levels of estradiol in serum (12), a second experiment was performed with a much lower dose of 10 μg of estradiol administered daily beginning 6 days before infection with yolk sac-grown GPIC. This estradiol regimen was found in previous experiments to produce estradiol levels in serum in the physiological range (9). The estradiol-treated group contained four animals, and the sesame oil-treated group had five. In this experiment, animals were inoculated ocularly with about 1.5×10^6 inclusion-forming units and in the genital tract with about 1.0×10^6 inclusion-forming units.

* Corresponding author.

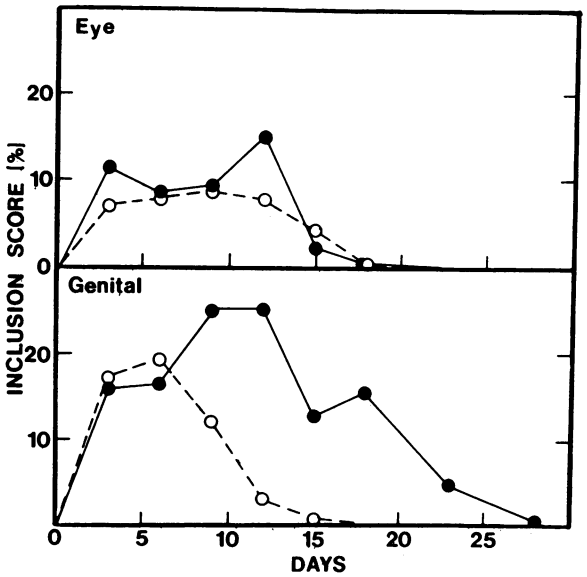

FIG. 1. Mean inclusion scores of animals infected ocularly or genitally with GPIC. Animals were treated with 1,000 μg of estradiol (●) or sesame oil (○).

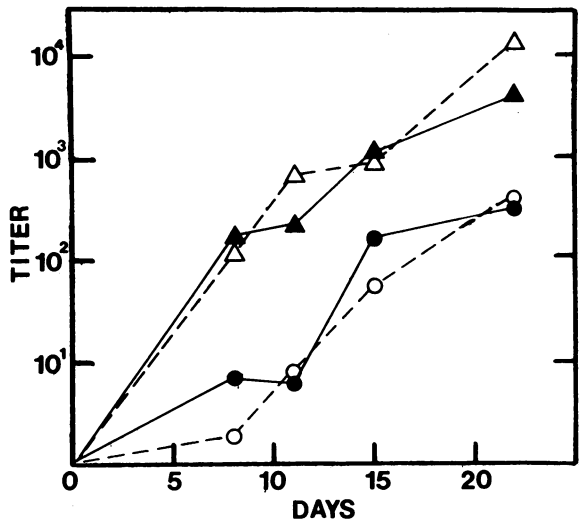

FIG. 2. Mean antibody titers in eye secretions of animals infected ocularly with GPIC and treated with 1,000 μg of estradiol or sesame oil. IgG titers in estradiol (●) and sesame oil-treated (○) guinea pigs. IgA titers in estradiol (▲)- and sesame oil (△)-treated guinea pigs.

When animals were treated with 1,000 μg of estradiol daily, there was no apparent effect on the course of ocular infection (Fig. 1). In contrast, genital tract infection was found to be significantly altered ($P < 0.005$ when the groups were compared by a two-factor [treatment group and days] analysis of variance with repeated measures on one factor [days]) in that the infection persisted at higher levels for a longer time and was extended by about 13 days, as was seen previously (12). Similarly, the lower-dose regimen of 10 μg of estradiol had no effect on the course of ocular infection but did significantly ($P < 0.002$) lengthen the course of genital infection by about 9 days.

IgG antibody levels in serum resulting from either genital or ocular infection were not affected by treatment with either 1,000 or 10 μg of estradiol. The response kinetics in animals infected in both sites and given either estradiol or sesame oil were virtually identical.

When IgG and IgA antibody titers to GPIC were determined in eye secretions during the course of ocular infection, no differences were seen between guinea pigs treated with 1,000 μg of estradiol and those treated with sesame oil alone (Fig. 2). Similar results were obtained when animals were treated with the 10-μg regimen. However, when genital secretions from the same animals were assessed for IgG and IgA, it was found that specific IgG did not appear during the observation time in animals treated with either 1,000 or 10 μg of estradiol (Fig. 3). These differences were significant in both experiments ($P < 0.02$ and $P < 0.002$, respectively). Unaccountably, specific IgA was only present at minimal levels in both estradiol-treated and control animals in the 1,000-μg experiment and was not obtained in the 10-μg experiment because of insufficient amounts of secretions. In a more recent experiment, IgA has been found to reach titers of 80 to 160 by day 28 in genital secretions as a result of ocular infection (Rank, unpublished data).

The results of estradiol treatment on the appearance of antibodies to GPIC in genital secretions during genital infection were also examined in this study as a control for comparison with previous experimentation. As was seen before (10), a marked delay in the development of both IgA ($P < 0.02$) and IgG ($P < 0.02$) was observed in animals treated with 1,000 μg and a delay in IgG was seen in animals treated with 10 μg. Insufficient secretions were available for determination of IgA titers in the latter group. In addition, eye secretions were examined in the genitally infected group to determine whether estradiol affected the levels of antibody at this site. Unfortunately, several of the animals receiving the 1,000-μg regimen developed ocular infections, so the data had to be discarded as unreliable. Animals

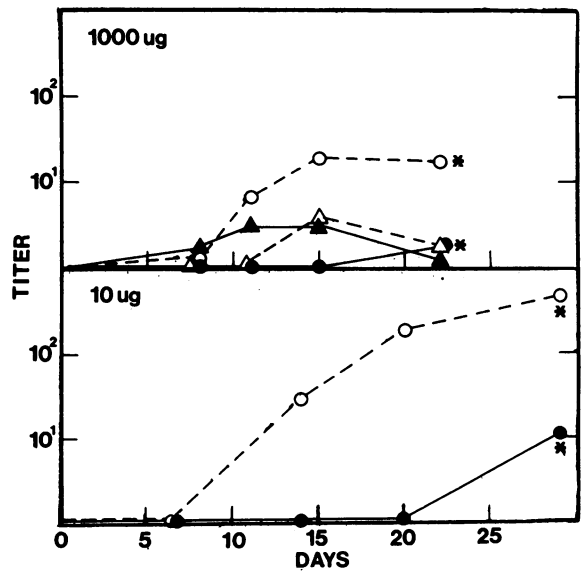

FIG. 3. Mean antibody titers in genital secretions of animals infected ocularly with GPIC and treated with 1,000 or 10 μg of estradiol. IgG titers in estradiol (●)- and sesame oil (○)-treated guinea pigs. IgA titers in estradiol (▲)- and sesame oil (△)-treated guinea pigs. Asterisks indicate significant differences between the estradiol and control groups.

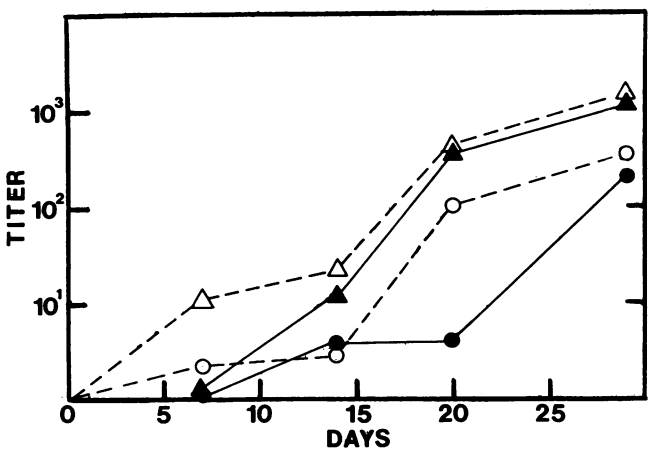

FIG. 4. Mean antibody titers in eye secretions of animals infeoted genitally with GPIC and treated with 10 μg of estradiol. IgG titers in estradiol (●)- and sesame oil (○)-treated guinea pigs. IgA titers in estradiol (▲)- and sesame oil (△)-treated guinea pigs.

receiving the 10-μg dose remained free of ocular infection so that ocular development of antibodies could be evaluated. Both IgG and IgA appeared ocularly as a consequence of genital infection, although IgG was somewhat delayed (Fig. 4). However, no statistically significant differences in the kinetics of the antibody response were noted.

The data presented in this study indicate that the ability of estradiol to alter the pathogenesis of chlamydial infection is restricted to infection of the genital tract and is not a generalized systemic phenomenon since ocular infection remained unaltered by estradiol treatment. These results are not completely unexpected since the female genital tract has estrogen receptors (5), whereas evidence for estrogen receptors on conjunctival cells has not been reported.

Of significance is our observation that delay in local antibody production was also restricted to the genital tract. Compatible with an earlier study F. R. Watson and E. S. Murray, Fed. Proc. 33:763, 1974), antibodies to GPIC were found in the corresponding distal mucosal secretions after either genital or ocular infection. However, estradiol induced a delay in the appearance of antibodies only in genital secretions, even when the site of infection was ocular. If the effect of estrogen were generalized to all mucosal tissues, one would expect some alteration of IgG and IgA development in eye secretions as a result of ocular infection. The role of local antibody in the resolution of genital tract infection is supported by this study since prolongation of infection was seen only in genital tract infections and appeared to be associated temporally with the delay in antibody production. It has been previously shown that cell-mediated immunity as measured by delayed-type hypersensitivity is apparently unaffected by estradiol treatment (12).

The reasons for the delay in antibody response in the genital tract are not clear. It could be related to physiological effects of estrogens on the genital tissues which result in increased cellularity, vascularity, and edema (6) and could potentially affect local antibody secretion. Alternatively, increased genital secretions resulting from estrogen influence (6, 13) may effectively dilute the antibody so that there is less antibody per volume of secretions available to combine with the organisms.

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