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Protective Role of Serum Antibody in Immunity to Chlamydial Genital Infection

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Female guinea pigs were injected intraperitoneally with pooled immunoglobulin derived from animals immunized to the chlamydial agent of guinea pig inclusion conjunctivitis. Genital infections in animals receiving pooled immunoglobulin from immune animals were markedly decreased with regard to the number of inclusions detected compared with control animals. These data indicated that serum-derived antibody was able to provide a degree of protection against a chlamydial genital tract infection.

Previous studies on the guinea pig model of chlamydial genital infection have indicated that both humoral and cell-mediated immunity are required for recovery from infection with the agent of guinea pig inclusion conjunctivitis (GPIC) and for resistance to reinfection (13, 14, 16). It has been assumed that since the infection is a local infection of a mucosal tissue, secretory immunoglobulin A (IgA) should be the major immunoglobulin isotype involved in the protective response. This assumption has been founded in observations made by Murray and colleagues in which newborn guinea pigs, having high levels of maternal-derived serum IgG, were not resistant to ocular infection with GPIC (9). Similarly, adult animals with high serum antibody levels as a result of artificial immunization but with no local antibody also lacked resistance to challenge infection. Moreover, passive immunization of guinea pigs (19) or owl monkeys (11) with immune serum did not provide any measurable degree of protection against ocular infection. However, in neither instance were antibodies detectable in eye secretions as a result of the passive transfer of serum. In contrast, a strong correlation was seen between the presence of secretory IgA in eye secretions and resistance to infection (10). In a slightly different study using the GPIC-guinea pig ocular model, Malaty et al. (6) found that resistance to a high-dose challenge infection, at various times after recovery from a primary infection, was correlated with increased antibody levels in both serum and secretions.

In genital tract infections, we have observed that the appearance of antibodies in serum and secretions is associated with recovery from the infection (12, 16). Evidence for the local action of antibody against chlamydiae in the genital tract was derived from a study in which the infection was prolonged by treatment with estradiol (12). This prolongation correlated with a delay in the production of antibody in secretions in the presence of a normal serum antibody response. The appearance of both IgA and IgG in secretions was delayed in hormone-treated animals. In general, there is no evidence which assigns a dominant protective function to either IgA or IgG in genital tract infections. Thus, it was the purpose of this investigation to determine whether serum-derived IgG in the genital tract could provide a protective role in resistance to chlamydial genital tract infection in the absence of local antibody production or cell-mediated immunity.

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Hartley strain female guinea pigs, weighing 450 to 500 g, were obtained from Sasco Laboratories, Omaha, Nebr., and were housed individually in an environmentally controlled room with a 12-h light-dark cycle. Guinea pigs were infected in the genital tract with GPIC which had been grown in HeLa cells (15). Each animal received approximately 1.4×10^7 inclusion-forming units contained in 0.05 ml of sucrose-phosphate-glutamate buffer (pH 7.4) (18). The course of the infection was monitored by determining the percentage of inclusion-bearing cells on a Giemsa-stained smear of a scraping from the vaginal vault (15). Antibody titers in serum and genital secretions were determined by an enzyme-linked immunosorbent assay, using GPIC elementary bodies grown in HeLa cells as the antigen (4). Immunoblot analyses were also performed as previously described (1).

The anti-GPIC immunoglobulin preparation was derived from pooled sera from guinea pigs from the same closed colony which had been immunized by either subcutaneous or intravenous routes with either viable or inactivated (UV light) organisms grown in McCoy cells. All immunized animals had been challenged with a viable genital tract infection with GPIC. The immunoglobulin fraction was obtained by precipitation with 50% ammonium sulfate. The fraction was redissolved in phosphate-buffered saline (pH 7.2), dialyzed, and filter sterilized. The immunoglobulin pool in experiment 1 contained 19 mg of protein per ml as determined by the Lowry protein assay (5) and had a titer of 2,560 to GPIC. The pool used in experiment 2 had 13 mg of protein per ml and a titer of 1,280 to GPIC. The antibody profile of the immunoglobulin pool, when assessed by immunoblot analysis, was the same as that of serum from animals which had recovered from a natural genital tract infection.

Two separate experiments were conducted in which five guinea pigs each were injected intraperitoneally with the pooled immunoglobulin while five animals remained untreated (experiment 1) or were injected with equivalent volumes of phosphate-buffered saline (experiment 2). Immunoglobulin treatment was begun several days prior to infection with GPIC so that high titers of antibody would be present in serum and genital secretions at the time of infection. In experiment 1, animals received 2 ml of the immunoglobulin on days −4 and −3 (with respect to the day of infection), 1 ml of immunoglobulin on days −2 to 1, and 0.5 ml of immunoglobulin on days 2 to 7. In experiment 2, animals were injected with 2 ml of immunoglobulin or

TABLE 1. Specific anti-GPIC antibody levels in serum and secretions of immune globulin recipients 1 day prior to GPIC infection

Experiment	Antibody	Antibody levels in:	
		Secretions (range [mean])	Serum (range [mean])
1	IgG	320–640 (368)	640–1280 (844)
	IgA	10–40 (26)	
2	IgG	40–640 (242)	1,280–2,560 (1,689)
	IgA	10–20 (17)	

phosphate-buffered saline on days −7 to 1 and 1 ml on days 2 to 7. Antibody titers were assessed on the day of infection in both serum and secretions. High titers of IgG specific for GPIC were observed in the sera of all treated guinea pigs (Table 1). Furthermore, high titers of IgG were present in genital secretions of all treated animals just before infection, indicating that serum antibodies had transudated into the lumen of the genital tract. The IgG titers in sera and secretions were equivalent to those normally found in convalescent guinea pigs after a chlamydial genital tract infection. Low levels of specific IgA were also detected in genital secretions. These were lower than IgA titers found in convalescent animals shortly after recovery from infection but comparable to titers several months after resolution (15). Genital secretions of control animals were negative for both IgG and IgA antibodies to GPIC.

Sera and secretions were also compared by immunoblot analysis, by using both anti-IgG and anti-IgA as probes, to determine whether all types of antibodies had passed into the secretions from the blood. The assay did, in fact, confirm that antibodies in the secretions gave the same profile on immunoblot analysis as did the sera from the same animals (data not shown). The profiles in the different animals were remarkably alike with regard to intensity and pattern. There is normally much more variability among animals which have had a natural infection. Both sera and secretions reflected the same profile as the donor immunoglobulin preparation. All of the major specificities previously described were present (1).

When the course of the infection was determined, the inclusion scores were significantly lower in the immunoglobulin recipient group for the first 9 days of the infection in both experiments (Fig. 1). As the infection in the control group began to wane, the infection curves grew closer, although the infection in the treated group persisted slightly longer than it did in the control group and was more elevated in the latter stages of the infection in the first experiment. In both experiments, the course of the infection of the immunoglobulin group was significantly different than it was in the control group when the data were compared by a two-factor (treatment group and days) analysis of variance with repeated measures on one variable (days) ($P < 0.001$).

These data indicate that serum antibodies, and in particular IgG, are able to influence the course of a chlamydial genital tract infection. However, while they can afford a marked degree of protection, they are unable to produce complete immunity. It is likely that locally produced IgA and cell-mediated immunity are also necessary to effect maximum protective capability. Some serum-derived IgA was present in secretions, but it was at considerably lower levels than the IgG. Data obtained in recent studies have supported an important role of IgG in secretions in the protective immune response. We have observed that while guinea pigs

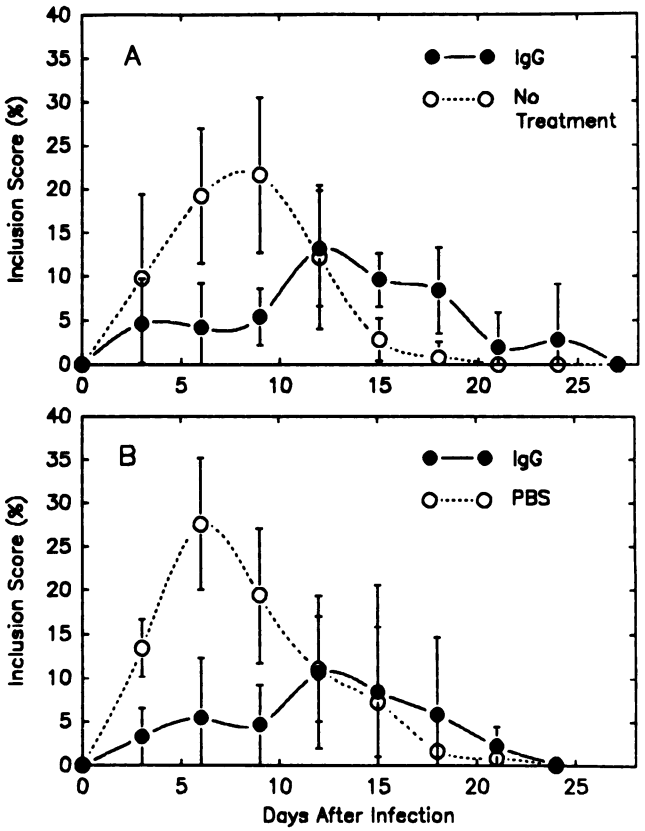

FIG. 1. Course of genital infections in guinea pigs treated with pooled immunoglobulin or phosphate-buffered saline or untreated. (A) Experiment 1. (B) Experiment 2. Error bars indicate the standard deviations of the means of the inclusion scores. PBS, Phosphate-buffered saline.

become susceptible to reinfection rather soon after resolution of a primary genital tract infection, they nonetheless do not develop a high level of infection (15). At these time points, specific IgA levels in secretions are quite low, but IgG levels are relatively elevated. Thus, serum-derived IgG in genital secretions may be an important antibody in controlling a challenge infection until anamnestic local IgA or cell-mediated immune responses or both can develop to halt the infection course.

In human chlamydial infections, anti-chlamydial antibodies of both IgG and IgA isotypes can be measured in cervicovaginal secretions (2, 8, 17). Richmond et al. (17) have suggested that the IgG is probably derived from serum by transudation. It has been noted that normal cervical secretions, unlike other mucosal secretions, have an IgA-IgG ratio of about 1:5, which approximates that of serum (7). This would imply that serum IgG is able to move into the genital tract with relative ease. The significance of the apparent protective role for serum antibody as demonstrated in the guinea pig model is that serum IgG may be a better predictor of protective immune status than is commonly thought. Indeed, Brunham et al. (3) reported that serum antibody titers were higher in women with chlamydial infections who did not develop postabortal salpingitis than in infected women who did develop postabortal salpingitis. They also related apparent protection to responsiveness to specific antigens. Thus, examination of sera of patients for antibodies to specific epitopes of the chlamydial outer membrane may yield valuable information regarding the immune

status of the patient. Moreover, these results suggest that parenteral routes of immunization which would preferentially elicit high levels of serum antibodies might indeed be practical and perhaps even desirable.

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