

## Humoral Immune Response in Acquired Immunity to Chlamydial Genital Infection of Female Guinea Pigs

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Immunity to reinfection in the genital tract of female guinea pigs with the agent of guinea pig inclusion conjunctivitis was found to be dependent upon an intact humoral immune response. Cell-mediated immunity in the absence of humoral immunity had no apparent role in resistance to challenge infection.

Presently, there is no firm evidence that *Chlamydia trachomatis* infections of the human genital tract elicit protective immunity to subsequent challenges. Such information is difficult to obtain in humans; however, the ability to infect guinea pigs in the genital tract with the agent of guinea pig inclusion conjunctivitis (GPIC), a *Chlamydia psittaci* organism (4), affords an excellent opportunity to investigate the mechanism by which protective immunity develops. Genital infection of female guinea pigs with GPIC lasts about 18 to 20 days and results in long-lasting immunity to challenge infection (1, 2, 5). We have previously shown that resolution of the infection is largely dependent upon humoral immunity (8); however, the nature of the immunity acquired against reinfection in this system is not known. Thus, it was the purpose of the present investigation to determine whether acquired immunity to GPIC is also dependent upon the humoral immune system.

Female Hartley strain guinea pigs weighing 400 to 500 g were obtained from Simonson Laboratories, Inc., Gilroy, Calif., and were housed individually with a fiber glass filter over each cage. This stock has been found to be free of GPIC infection; nevertheless, all animals were assessed for antibodies to GPIC before being included in the experiments. Guinea pigs were infected by intravaginal inoculation of 0.05 ml of a suspension containing approximately  $4.6 \times 10^5$  50% egg lethal doses derived from yolk sac-grown material as described previously (8). The course of the infection was assessed by scraping the vaginal wall with a dental spatula and making a smear on a glass slide. Smears were Giemsa stained after methanol fixation. A total of 100 epithelial cells were counted, and the percentage containing inclusions was recorded as the inclusion score (8).

Antibodies to GPIC in serum and genital secretions were detected by indirect immunofluo-

rescence with fluorescein-labeled rabbit anti-guinea pig immunoglobulin G (Miles Laboratories, Elkhart, Ind.) (8). Genital secretions were obtained by a modification of the procedure of Lamont et al. (2). A surgical sponge (Weck-Cel; Edward Weck and Co., Inc., Durham, N.C.) (2 by 10 mm) was inserted into the vagina of an animal anesthetized with Nembutal (Abbott Laboratories, North Chicago, Ill.) and was retrieved 2 h later. The sponge was weighed before and after insertion, and the weight of the collected secretion was determined. Immediately before antibody testing, the sponge was eluted in phosphate-buffered saline, pH 7.2, at a ratio of 0.2 ml of phosphate-buffered saline to 0.1 g of secretion. This solution was considered undiluted with regard to subsequent titration.

Allergic contact dermatitis to oxazolone and delayed-type hypersensitivity to GPIC antigen were performed as before (8, 9). Briefly, animals were sensitized with 0.2 ml of 10% oxazolone in acetone on the ear and were challenged on the flank with 0.125% oxazolone in acetone-corn oil (4:1). Reactions were graded according to intensity of erythema and induration 24 h after challenge. Delayed-type hypersensitivity to heat-killed GPIC antigen was assessed by injecting 0.05 ml of antigen prepared from GPIC grown in cell culture into the pinna of the ear and measuring the increase in ear thickness 24 h after challenge.

To determine whether humoral immunity is required for resistance to reinfection, we attempted to create a situation in which we could challenge animals which had active cell-mediated immunity (CMI) to GPIC but negligible humoral immunity. To do this, we treated one group (Cy-GPIC-Tc) of nine guinea pigs with 200 mg of cyclophosphamide (Fairfield Chemical Co., Inc., Blythewood, S.C.) per kg intraperitoneally on days 1, 9, 18, etc. for the course of the

experiment. This regimen has been shown to suppress humoral immunity but not CMI (8). Since GPIC infection does not resolve in cyclophosphamide-treated animals, we treated all animals with 10 mg of oxytetracycline (Rachelle Laboratories, Inc., Long Beach, Calif.) per kg intramuscularly twice daily for five consecutive days beginning on day 13 to eliminate the organism. After the infection had resolved as assessed by inclusion scores, the animals were challenged on day 24 with a GPIC suspension containing  $4.6 \times 10^5$  50% egg lethal doses. A control group (GPIC-Tc) of three animals was infected with GPIC and treated with tetracycline but did not receive cyclophosphamide. These animals would have recovered without tetracycline treatment. Finally, a third group (Tc) of three guinea pigs remained uninfected but was treated with tetracycline to verify that any potential residual levels of tetracycline would not interfere at the time of challenge infection. In addition, this group was not sensitized to oxazolone so that it could act as a negative control for this response.

All guinea pigs became infected upon inoculation with GPIC and were cured of the infection by treatment with tetracycline as indicated by negative inclusion scores on days 21 and 24 (Fig. 1). Interestingly, three Cy-GPIC-Tc animals in which negative inclusion scores on day 21 indicated an apparent cure redeveloped positive inclusion scores by day 24, indicating a resurgence or relapse of the infections. These animals

TABLE 1. CMI responses at time of challenge infection (day 24)

Group	Response (no. positive/no. tested) to:		Ear thickness ( $\times 0.1$ mm $\pm$ SD)
	Oxazolone	GPIC	
Cy-GPIC-Tc	6/6	6/6	$5.3 \pm 2.0$
GPIC-Tc	2/3	3/3	$5.8 \pm 1.8$
Tc	0/4	0/4	$0.8 \pm 0.6$

were excluded from the challenge experiment. When challenged on day 24 with a fresh suspension of GPIC, all six of the remaining Cy-GPIC-Tc guinea pigs became reinfected. In contrast, none of the immunologically intact animals (GPIC-Tc) became infected upon challenge and were thus judged to be immune. All of the animals in the Tc group were susceptible to the challenge infection (data not shown), confirming that residual drug was not playing any role in resistance. Combining data from other experiments showed that a total of 10 out of 10 Cy-GPIC-Tc animals became infected upon challenge, whereas 0 out of 9 GPIC-Tc guinea pigs became infected.

When the humoral immune responses of the various groups were assessed, the Cy-GPIC-Tc group had negligible levels of serum antibody to GPIC, whereas GPIC-Tc animals developed substantial titers by day 14 (Fig. 1). Similarly, Cy-GPIC-Tc animals had a mean secretion antibody titer of 1.2 in contrast to a mean titer of 64 in the GPIC-Tc animals. However, both the Cy-GPIC-Tc and the GPIC-Tc groups responded with significant delayed-type hypersensitivity reactions to GPIC antigen ( $P < 0.001$ ) on day 24 when compared with the Tc group by a two-tailed *t* test (Table 1). It is important to note that there was no significant difference between the Cy-GPIC-Tc and GPIC-Tc groups. Both groups also responded to challenge with oxazolone.

Thus, these data demonstrate that the Cy-GPIC-Tc group had developed strong CMI to GPIC as well as to a heterologous antigen but remained essentially negative for antibody production to GPIC in both serum and secretions. Despite the CMI to GPIC at the time of challenge infection, the Cy-GPIC-Tc group developed a second infection, suggesting that CMI is not responsible for acquired immunity to chlamydial genital infection. This study strongly indicates that resistance to challenge infection as well as resolution of primary infection is dependent upon the humoral immune response.

Although the particular class of immunoglobulin responsible for the protective immunity was not determined in this study, it seems likely that protection is associated with antibodies found in genital secretions, in particular with secretory

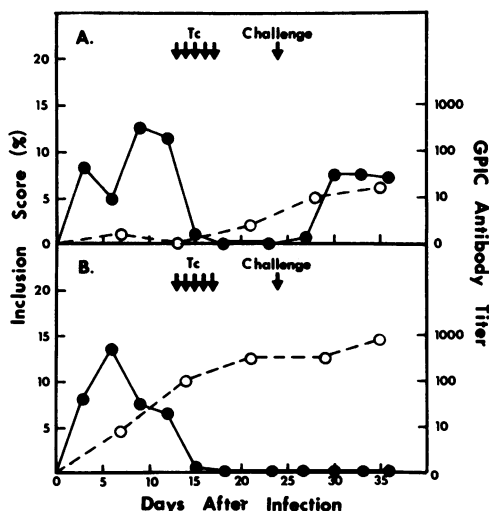


FIG. 1. Course of GPIC infections in the Cy-GPIC-Tc group (A) and the GPIC-Tc group (B) and results of challenge infections on day 24. Data points represent mean (arithmetic) inclusion scores (●) or mean (geometric) antibody titers to GPIC (○). Arrows show days of tetracycline treatment (Tc) and challenge.

immunoglobulin A. Secretory immunity has been implicated in the protective immune response to GPIC infections of the eye (3, 5-7) and genital tract (5). In contrast, the production of high levels of serum antibodies failed to provide immunity to local challenge with GPIC in the eye (6). Nichols et al. (7) immunized guinea pigs with viable GPIC orally and observed protection against challenge in the genital tract. They suggested that this immunity was associated with the development of a secretory immune response resulting from the oral immunization.

The finding that CMI does not play a significant role in resistance to challenge infection does not rule out the participation of T cells in this response. In fact, it is quite likely that T helper cells are essential for the production of antibody to GPIC. Williams et al. (10) have found that respiratory infections of mice with the agent of mouse pneumonitis (*C. trachomatis*) are more severe in congenitally athymic mice than in immunologically intact mice. However, this phenomenon appears to be related to the inability of the mice to form T cell-dependent antibodies rather than to the absence of CMI mechanisms.

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