INFECTION AND IMMUNITY, Aug. 1983, p. 876–879
0019-9567/83/080876-04$02.00/0

Vol. 41, No. 2

Effect of Antithymocyte Serum on the Course of Chlamydial Genital Infection in Female Guinea Pigs

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Received 10 March 1983/Accepted 23 May 1983

The treatment of female guinea pigs, infected in the genital tract with the chlamydial agent of guinea pig inclusion conjunctivitis, with rabbit anti-guinea pig thymocyte serum extended the course of the infection by 20 to 30 days. The rabbit anti-guinea pig thymocyte serum was shown to suppress delayed hypersensitivity responses to the guinea pig inclusion conjunctivitis agent and the contact allergen oxazolone. The appearance of antibody in genital secretions was delayed, but the infection persisted at low levels even when normal serum and secretory antibody titers were attained, indicating that cell-mediated immunity may play a role in the resolution of chlamydial genital infections.

In previous studies on the immune response to the agent of guinea pig inclusion conjunctivitis (GPIC), the development of humoral immunity has been identified as necessary for both resolution of and resistance to chlamydial genital infections (4, 5). Those studies also indicated that cell-mediated immunity (CMI) alone was unable to resolve the infection or provide immunity to challenge infection. However, the participation of CMI in concert with the humoral immune response was not ruled out. Thus, in the present investigation, we examined the role of CMI by treating female guinea pigs with rabbit anti-guinea pig thymocyte serum (ATS) during genital infection with GPIC.

Female Hartley strain guinea pigs weighing 400 to 500 g were obtained from Simonsen Laboratories, Inc., Gilroy, Calif., and were found to be GPIC-free as determined by an assessment of serum for antibodies to GPIC. Animals were housed individually in cages fitted with fiber glass filter tops. Guinea pigs were infected by intravaginal inoculation of 0.05 ml of a suspension containing either 7.9×10^5 (experiment 1) or 1.2×10^6 (experiment 2) 50% egg lethal doses derived from yolk-sac-grown material as described previously (7). The course of the infection was assessed by determining the percentage of epithelial cells containing inclusions in Giemsa-stained smears of vaginal wall scrapings. This percentage is referred to as the inclusion score.

Antibodies to GPIC in serum were measured by indirect microimmunofluorescence with fluorescein-labeled rabbit anti-guinea pig immunoglobulin G (IgG) (H and L chain; Miles Laboratories, Inc., Elkhart, Ind.) (5). Antibodies to GPIC in genital secretions were measured similarly; however, in addition, specific IgA antibodies to GPIC were determined in secretions by using rabbit anti-guinea pig IgA (alpha chain specific) followed by fluorescein-labeled goat anti-rabbit IgG (H and L chain) (Miles Laboratories). Genital secretions were obtained as described previously (4).

CMI was assessed by determining the contact allergic response to oxazolone (5) and the delayed-type hypersensitivity skin reaction to GPIC antigen (6). Briefly, animals were sensitized with 0.2 ml of 10% oxazolone in acetone on the ear on the day of GPIC inoculation. They were challenged 7 days later on the flank with 0.125% oxazolone in acetone-corn oil (4:1). Reactions were graded according to the intensity of erythema and edema 24 h after challenge. Delayed-type hypersensitivity to heat-killed GPIC antigen prepared from GPIC grown in BGM cell cultures (5×10^6 inclusion-forming units per ml) was assessed by injecting 0.05 ml of antigen into the pinna of the ear and measuring the increase in ear thickness 24 h after challenge.

ATS was prepared as follows. Thymuses were removed from 1-week-old outbred guinea pigs and minced into a single cell suspension in phosphate-buffered saline (pH 7.2) containing 0.1% EDTA. The cell suspension was washed three times in phosphate-buffered saline-EDTA by centrifugation at $192 \times g$, and the cell pellet was suspended in equal parts of phosphate-buffered saline and Tris-ammonium chloride to lyse erythrocytes (1). The remaining thymocytes were washed an additional three times and were suspended in phosphate-buffered saline so that 1 ml contained ca. 10^8 cells. Equal parts of the cell

suspension and Freund complete adjuvant (GIBCO Laboratories, Grand Island, N.Y.) were emulsified, and 1 ml of the final mixture containing 5×10^7 thymocytes was injected intradermally at five sites (0.2 ml per site) on the back of a New Zealand white rabbit. Two booster inoculations of 5×10^7 thymocytes were given intravenously at 2-week intervals, followed by similar boosters at 1-month intervals thereafter.

Blood was collected at various times after the initial two booster inoculations. Before experimental use, the ATS was heat-inactivated (56°C for 30 min) and absorbed at least twice with 10% fresh washed guinea pig erythrocytes. The titer of the ATS was determined by agglutination of fresh guinea pig thymocytes (8). The titers of the sera used ranged from 160 to 320. Normal rabbit serum (NRS) was heat-inactivated and absorbed with guinea pig erythrocytes if antibodies to guinea pig erythrocytes were present. Guinea pigs were injected daily with 1 ml of ATS or NRS beginning 1 day before infection with GPIC and continuing throughout the experiment.

Two separate experiments were performed in which animals treated with either ATS or NRS were inoculated intravaginally with GPIC. In experiment 1, the ATS group contained five guinea pigs, and the NRS group contained three, whereas in experiment 2, the groups consisted of six and five animals, respectively. In both experiments, the initial stage of infection up to day 15 was similar in both ATS- and NRS-treated animals (Fig. 1). However, while the infections in NRS-treated guinea pigs were resolving, elevated inclusion scores were maintained in the ATS-treated animals until days 20 to 25, when the scores then dropped to levels ≤5%. The course of infection in NRS-treated animals was characteristic of a normal GPIC genital infection. In experiment 1, the infections in ATS-treated animals finally resolved by day 41 but did not resolve until day 54 in experiment 2. In experiment 2, ATS treatment was stopped on day 52 due to exhaustion of the ATS supply. In both experiments, the course of infection in ATS-treated animals was significantly different from that in the NRS-treated animals when the curves were compared by a two-factor (treatment group and days) analysis of variance with repeated measures on one factor (days). The results in experiment 1 were $F(1,6) = 15.96$ and $P < 0.007$, and in experiment 2 they were $F(1,9) = 16.35$ and $P < 0.003$.

To demonstrate that the ATS was indeed suppressing the CMI response, each animal was tested for its responsiveness to oxazolone on day 7 and to GPIC antigen on day 14 (experiment 1) or day 15 (experiment 2). The results indicated that ATS did in fact suppress CMI

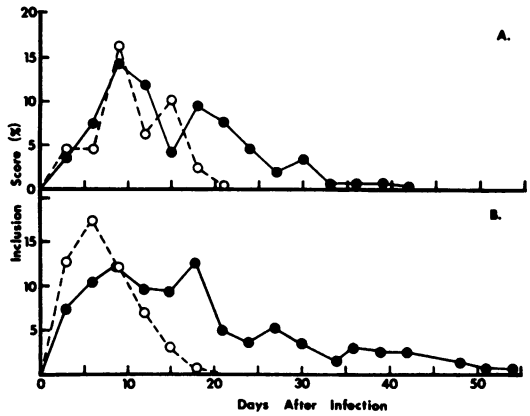

FIG. 1. Course of GPIC genital infection in guinea pigs treated with either ATS (●) or NRS (○). A, Experiment 1; B, experiment 2.

responses in most guinea pigs (Table 1). In each experiment, one animal was not completely suppressed, as indicated by a response to one antigen but not the other. In contrast, most animals in the NRS groups had positive responses.

Serum antibodies to GPIC were first detected on day 14 in both ATS and NRS groups and rose to high titers by day 21 (Table 2). The antibody responses were similar in both groups.

Antibodies in genital secretions were determined in experiment 2. In contrast to the serum antibody response, a marked difference was noted in the appearance of both total antibodies and specific IgA antibodies to GPIC in genital secretions (Table 3). Both total and IgA antibodies appeared more slowly in ATS-treated animals than in the NRS-treated animals and did not attain equivalent titers until day 33. Substantial titers of specific IgA were detected on day 19 in NRS-treated animals. IgA titers of the ATS group were significantly lower than those of the NRS group on days 19 and 26 ($P < 0.001$ and $P < 0.01$, respectively, according to a one-tailed t test), whereas total antibody titers were significantly lower only on day 19 ($P < 0.001$). The appearance of higher titers corresponded with

TABLE 1. CMI responses in guinea pigs treated with ATS and NRS

Expt	Treatment	Response[a] with:		Ear thickness (×0.1 mm ± SD)
		Oxazolone	GPIC	
1	NRS	2/3	3/3	7.5 ± 3.0
	ATS	0/5	1/5	0.8 ± 0.5
2	NRS	5/5	4/5	12.5 ± 1.3
	ATS	1/6	0/6	0.9 ± 0.4

[a] Number positive/number tested.

TABLE 2. Antibody response to GPIC in serum in guinea pigs treated with ATS and NRS

Expt	Treatment	Antibody titer (log 10) at the following days after infection							
		7	14	21	28	35	42	49	61
1	NRS	<1.0	1.8 (0.2)[a]	2.3 (0.2)	2.3 (0.2)	2.4 (0.2)	2.1 (0.2)		
	ATS	<1.0	1.7 (0.3)	2.4 (0.2)	2.7 (0.5)	2.4 (1.3)	2.9 (0.5)		
2	NRS	<1.0 (0.3)	1.9 (0.1)	2.6 (0.2)	2.5 (0.2)	2.4 (0.2)	2.1 (0.2)	2.4 (0.2)	2.9 (0.3)
	ATS	<1.0 (0.5)	1.6 (0.3)	2.3 (0.3)	2.7 (0.3)	3.0 (0.2)	3.1 (0.3)	3.1 (0.3)	3.2 (0.2)

[a] Numbers in parentheses are standard deviations.

the decrease in inclusion scores of ATS-treated guinea pigs (Fig. 1).

There are two possible mechanisms for the prolonged GPIC infection caused by treatment with ATS, and it seems feasible that both may be operative in this model. According to the first mechanism, T helper cells necessary to aid in the production of secretory IgA may have been eliminated in part, thereby delaying the production of secretory antibody. It is interesting to note that the decrease in inclusion scores on days 20 to 25 coincided with an increase in the secretory antibody levels. It is assumed that secretory IgA plays a major role in the resolution of GPIC genital infections although it has not been specifically proven. Immunization of guinea pigs by the oral route can elicit protective immunity in the genital tract, presumably by the circulation of sensitized cells in the mucosal-associated lymphoid tissue (3). Williams et al. reported that respiratory infections with the agent of mouse pneumonitis (*Chlamydia trachomatis*) produced lethal infections in congenitally athymic nude mice (11). Based on an absence of specific antibody in these mice and the protection of the mice with immune serum, Williams et al. suggested that it was the lack of T cell-dependent antibody production in the nude mice that caused the lethal infection (13).

It was surprising to us that there was no apparent effect of ATS on the production of serum antibody as one would expect if indeed T helper cells were being affected. Schell et al. (8, 9) also attempted to suppress the antibody response to sheep erythrocytes in guinea pigs with antilymphocyte serum and ATS. Although they could suppress CMI responses, they were also unsuccessful in altering the antibody response. The reason for this phenomenon is unknown.

Of great interest was the observation that the infection in ATS-treated animals was significantly prolonged, even in the presence of substantial titers of serum and secretory antibodies. These data indicate that CMI may play a role in effecting complete resolution of GPIC genital infection. Since previous studies showed that humoral immunity is required for the resolution of GPIC genital infection and that CMI in the absence of a humoral response cannot resolve the infection (5), the data presented here suggest that both a humoral and a CMI response are required to resolve the infection. These findings support an earlier study (J. D. Mull and S. E. Thompson III, Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, V33, p. 200) in which a GPIC conjunctival infection was prolonged by treatment with antilymphocyte globulin, also in the presence of normal serum and eye secretion antibody titers. In that study, CMI was shown to be absent by negative responses to skin tests with purified protein derivative after immunization with BCG. Furthermore, Williams et al. (12) indicated in a preliminary experiment that CMI might be involved in a protective role in respiratory infections of nude mice with the agent of mouse pneumonitis. They observed that nude mice receiving T cell-depleted immune spleen cells had higher mortality than recipients of T

TABLE 3. Antibody response to GPIC in genital secretions in guinea pigs treated with ATS and NRS

Antibody	Treatment	Antibody titer (log 10) at the following days after infection					
		19	26	33	40	47	61
IgA	NRS	1.9 (0.2)[a]	1.9 (0.1)	1.4 (0.3)	1.5 (0.2)	1.4 (0.4)	1.4 (0.3)
	ATS	0.2 (0.4)	0.8 (0.8)	1.4 (0.5)	1.9 (0.5)	1.9 (0.3)	2.1 (0.3)
Total immunoglobulin	NRS	1.6 (0.4)	1.5 (0.4)	1.2 (0.4)	1.4 (0.3)	1.1 (0.3)	1.6 (0.3)
	ATS	0.3 (0.3)	1.1 (0.7)	1.7 (0.3)	2.0 (0.3)	2.3 (0.2)	2.2 (0.2)

[a] Numbers in parentheses are the standard deviations.

cell-enriched populations, although both groups developed specific IgG and IgA. Kuo and Chen (2) have also reported that the resolution of respiratory infections in mice with ocular and genital strains of *C. trachomatis* seems to correlate with the appearance of delayed-type hypersensitivity. In contrast, Tuffrey et al. (10) reported that the genital infection of nude mice with the SA-2f serotype of *C. trachomatis* was self-limited, with no significant difference in the length of infection between the nude and normal mice. Thus, they suggested that T cell-dependent mechanisms are of minimal importance in the recovery of mice from chlamydial genital infection in the system they employed.

We thank Lisa Kelly and Theresa Dunn for their excellent technical assistance.

This investigation was supported by Public Health Service grant AI 13069 from the National Institute of Allergy and Infectious Diseases.

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