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Intrarectal Infection of Guinea Pigs with the Agent of Guinea Pig Inclusion Conjunctivitis[1] (39552)

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Chlamydial infection of the human genital tract is a topic of current concern (1). A well-known venereal disease caused by a chlamydial agent is lymphogranuloma venereum (LGV). The association of chlamydiae with human urethritis (2) and cervicitis (3) is a subject of great interest. Chlamydiae have been recovered from the rectum as well as from the genital tract (4). Organisms causing human genital infection as well as ocular disease are classified in Group A (*Chlamydia trachomatis*). In general, chlamydial infections occurring in other animals are caused by organisms in Group B (*Chlamydia psittaci*).

In our laboratory, we have developed the guinea pig as an animal model system for the investigation of chlamydial genital infections using the agent of guinea pig inclusion conjunctivitis (Gp-ic) discovered by Murray in 1964 (5). In the natural host, this organism causes a mild conjunctivitis. It has been shown that experimentally infected female guinea pigs develop vaginitis and cervicitis and are capable of transmitting ocular infection to newborns (6). The male guinea pig can be infected experimentally by the intraurethral route, and subsequent transmission of genital infection to females has been demonstrated (7).

In our former studies, leg lesions were observed in male guinea pigs inoculated intraurethrally (8). These lesions may now be accounted for by the use of Innovar-vet as an analgesic tranquilizer since this drug has been shown to cause self-mutilation in guinea pigs (9).

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In view of the possible significance of rectal infection in man caused by chlamydiae, we have investigated experimental infection of guinea pigs by the intrarectal route with the agent of Gp-ic. The results are presented in this report.

Materials and methods. Methods for preparation of inoculum from Gp-ic-infected yolk sac suspensions, collection of vaginal and conjunctival scrapings for smears stained with Giemsa, detection of antibodies by indirect immunofluorescence have been described previously (6, 7).

Guinea pigs. Mature Hartley strain guinea pigs were obtained from Simonsen Laboratories, Inc., Gilroy, Calif. All animals were pretested in our laboratory for antibodies to Gp-ic. Conjunctival and vaginal smears were also examined for Gp-ic inclusions, and no evidence was obtained for previous or current infection. Animals were caged individually for these experiments.

Intrarectal inoculation. All animals were fasted overnight before inoculation. A sterile vinyl tubing attached to a 23-gauge needle on a 1.0-ml syringe was inserted about 1.5 in. (3.8 cm) into the rectum. Each animal received 0.25 ml of inoculum containing approximately 2×10^6 ELD_{50}. Leakage from the rectum occurred in most instances so that exact volume retained was not determined.

Rectal swabs and smears. A dental spatula was inserted into the rectum and scraped over the intestinal wall. Sterile cotton swabs were inserted into the rectum and gently rotated. The swabs were placed in 1.0 ml of SPG (sucrose-potassium-glutamate) containing streptomycin (2.5 mg/ml) and vancomycin (0.5 mg/ml). Samples were frozen at −70°C until used for inoculation of chick embryos.

Isolation of Gp-ic from intestinal seg-

ments. Animals were anaesthetized with ether and exsanquinated by cardiac puncture. A rectal swab was obtained and the peritoneum was opened using sterile technique. The intestine was severed at the most distal point within the pelvis and dissected on a sheet of sterile aluminum foil. Segments were removed at intervals of approximately 3 in. (7.6 cm) and the contents were expressed onto the foil. One part of each level was placed in SPG and frozen at −70°C for homogenization and inoculation into chick embryos. An adjacent segment was collected for histopathology.

Results. The pattern of recovery of Gp-ic after inoculating male and female guinea pigs intrarectally is shown in Fig. 1. Gp-ic was recovered from all animals on Day 7 postinoculation and from 10 of 14 on Day 10, indicating that all animals were successfully infected. By Day 14, six of eight males were still shedding the agent whereas only one female of seven was positive. After this period, Gp-ic was recovered from only a few animals. One male (no. 317) was still positive on Day 35, but a specimen collected on Day 42 yielded negative results. In several animals, a sporadic pattern of isolation was observed and it is not known if this type of response was due to inadequate collection of specimens or sporadic shedding of the agent.

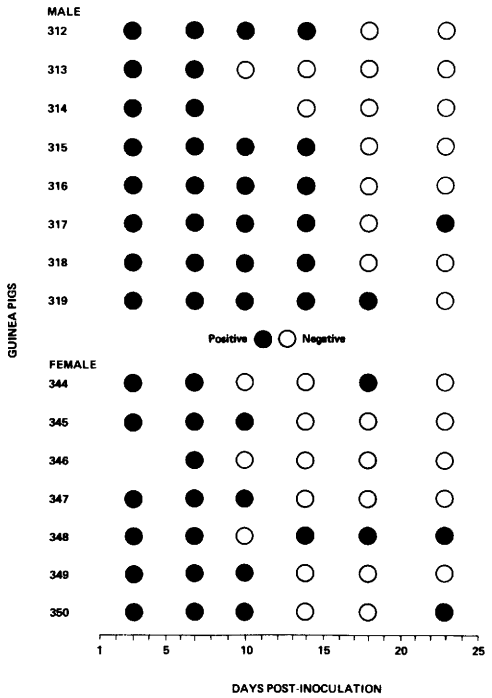

FIG. 1. Recovery of Gp-ic agent from guinea pigs inoculated intrarectally. Rectal swabs were collected and inoculated into 7-day-old chick embryos.

Attempts were made to detect Gp-ic inclusions in rectal smears stained with Giemsa. Most of the specimens were inadequate for evaluation but in two animals (male no. 319, female no. 347) sufficient cellular material was obtained and Gp-ic inclusions were observed in cells. The guinea pigs were also examined for overt clinical response with negative results. Rectal temperatures were followed for 7 days postinoculation with no elevations observed.

As shown in Table I, antibody responses were detected following intrarectal inoculation with Gp-ic. All animals had been pretested prior to inoculation and all sera were negative at a dilution of 1:10. Antibodies were detected in all animals except male no. 319 which remained negative throughout the course of the experiment. Titers were generally in the range of 10–20, and by Day 42, 5 of the 15 animals yielded negative

TABLE I. ANTIBODY RESPONSE IN GUINEA PIGS INOCULATED INTRARECTALLY WITH Gp-ic.

	Day postinoculation		
Guinea pigs	21	42	77
Male			
312[a]	20[b]	40	10
313	20	10	<10
314[a]	10	<10	<10
315[a]	10	10	<10
316	10	<10	<10
317	10	<10	<10
318[a]	20	10	<10
319	<10	<10	<10
Female			
344	20	ND[c]	<10
345[a]	20	20	<10
346	10	<10	<10
347[d]	<10	10	<10
348	40	80	10
349	20	40	<10
350	20	40	10

[a] Conjunctival smear positive for chlamydial inclusions.

[b] Antibody titer determined by indirect immunofluorescence; see Materials and Methods.

[c] Not done.

[d] Vaginal smear positive for chlamydial inclusions.

results. Only 3 of the 15 animals had detectable antibodies by Day 77.

In order to learn the extent of spread of Gp-ic in the intestinal tract following intrarectal inoculation, groups of male and female guinea pigs were inoculated and sacrificed on selected days and the intestinal tract was dissected (see Materials and Methods). Rectal swabs from 16 of 20 animals tested on the day of sacrifice were positive for Gp-ic (Table II). Homogenates of rectal tissue yielded Gp-ic from 12 of the 21 animals. In considering the possible migration of the infection in the intestine, combination of results obtained from section nos. 4, 5, and 6 (Table II) revealed that Gp-ic was not isolated from these sites on either Day 3 or Day 7. However, from Days 10 to 21, the agent was recovered from these sites. It was not apparent that Gp-ic was uniformly distributed throughout the intestinal tract. Failure to recover the agent from adjacent sections may have been due to technical error or may indicate a patchy localization of the agent. None of the serum specimens collected on date of sacrifice in this experiment yielded positive results for Gp-ic antibodies. Histologic sections of the intestinal segments did not reveal any obvious pathologic process and Gp-ic inclusions were not observed in superficial epithelium.

It was also of interest to examine the incidence of ocular and genital infections following intrarectal inoculation. In this study, a total of 8 animals of 35 tested yielded ocular smears that were positive for Gp-ic inclusions. Inclusions were also detected in vaginal smears of 2 of 16 females.

Discussion. Male to female transmission of Gp-ic by sexual contact has been documented under experimental conditions (7), but data have yet to be obtained for natural genital infection of either males or females. The demonstration of experimental intrarectal infection of guinea pigs with Gp-ic broadens the potential use of this *Chlamydia*-host system for the study of genital infections caused by these agents. No obvious clinical condition or histopathologic response following rectal infection was ob-

TABLE II. RECOVERY OF Gp-ic FROM INTESTINAL TRACT OF GUINEA PIGS INOCULATED INTRARECTALLY.

Guinea pig		Intestinal segments							
		Rectal[a] swab	Rectum	1[b]	2	3	4	5	6
Day 3	M-352	+[c]	+	–	–	C[d]	–	–	–
	M-353	+	+	+	+	–	–	–	–
	F-364	–	+	+	–	–	–	–	–
	F-366	–	–	–	–	–	–	–	–
Day 7	M-354	+	+	–	–	–	–	–	–
	M-355	+	–	–	–	–	–	–	–
	F-367[e]	+	+	C	+	–	–	–	–
	F-368	+	+	+	–	–	–	–	–
Day	M-356	+	–	–	–	–	–	–	–
10	M-357	–	+	–	–	–	–	+	–
	F-369	+	–	–	–	–	–	–	–
	F-379	+	+	–	–	–	–	–	+
Day	M-359[f]	+	–	–	+	–	–	+	–
14	M-360	+	+	–	+	–	–	–	–
	F-371	C	+	+	–	+	+	+	–
	F-372[f]	+	–	–	–	–	–	–	–
Day	M-358	+	+	–	–	–	–	–	–
17	M-361[f]	+	–	–	–	+	–	–	+
	M-362	+	–	–	–	–	–	–	–
	F-374	+	+	–	–	–	–	ND[g]	–

[a] Collected on day of sacrifice.
[b] Most distal segment of colon from abdomen.
[c] Specimens were inoculated into chick embryos via yolk sac route.
[d] Contaminated.
[e] Vaginal smear positive for chlamydial inclusions.
[f] Conjunctival smear positive for chlamydial inclusions.
[g] Not done.

served. Our experiments showed that shedding of Gp-ic could be demonstrated in males and females for at least 10 days following intrarectal inoculation. After this time, the incidence was apparently lower in females than in males. Recovery of Gp-ic from upper segments of the intestinal tract indicates the potential for continued infection. In this regard, it is also possible that contamination of food and cages with fecal material led to infection by fecal-oral route. No data are available on this potential route.

Immune response was documented in animals inoculated intrarectally. Antibody titers were somewhat lower than those observed in females inoculated intravaginally (6) or males inoculated intraurethrally (7). Interpretation of the antibody response following intrarectal inoculation is complicated by the fact that a number of animals was also infected in the eye and in the genital tract. One might have expected that in these instances an antibody response compatible with higher titers previously obtained following infection at these sites would have resulted. However, the antibody response may have been influenced by the time course of infection as related to collection of sera.

Summary. Evidence for infection of male and female guinea pigs by the chlamydial agent of guinea pig inclusion conjunctivitis (Gp-ic) following intrarectal inoculation was documented by recovery of the agent from rectal swabs up to Day 23 and from segments of intestinal tract to Day 17. Antibody response to Gp-ic was demonstrated in the serum by Day 21 although titers were low as detected by indirect immunofluorescence. In a few instances scrapings of intestinal tissue were found to contain cells with Gp-ic inclusions when stained with Giemsa. Gp-ic inclusions were also observed in ocular smears from males and females (8 positive/35 tested) as well as vaginal smears (2 positive/16 tested). These studies augment the value of this animal model system for the study of genital infections caused by *Chlamydia*.

This paper is devoted to our friend Doctor Felix Milgrom of the State University of New York at Buffalo on the occasion of the thirtieth anniversary of his research activities.

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