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Experimental Genital Infection of Male Guinea Pigs with the Agent of Guinea Pig Inclusion Conjunctivitis and Transmission to Females

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Male guinea pigs were inoculated intraurethrally with the agent of guinea pig inclusion conjunctivitis (Gp-ic). Cytoplasmic inclusions were found in superficial epithelial cells of the urethra in smears and stained sections. Gp-ic antigen(s) was detected by immunofluorescent staining of sections. There was no marked urethral exudate, but many animals developed bullous lesions on the glans and the body of the penis and a severe inflammatory lesion of the hind leg. All males demonstrated an antibody response and most of them showed a positive skin test reaction. Venereal transmission to females of Gp-ic infection was shown to occur as determined by detection of inclusions in vaginal smears, antibody response, and positive skin tests.

Lymphogranuloma venereum, which has long been recognized as an important venereal disease, is caused by a chlamydial agent. Interest in the role of other chlamydiae in human genital tract infections has recently increased. There is now ample evidence for the implication of trachoma-inclusion conjunctivitis (TRIC) agents in cervicitis, urethritis, and proctitis (1). An animal model system for the study of pathogenicity, transmission, and immunity in chlamydial genital infections would be very useful. The agent of guinea pig inclusion conjunctivitis (Gp-ic) which belongs to the *Chlamydia* group was first described by Murray (4) as the cause of a mild ocular disease of young animals. We reported previously on the experimental genital tract infection of female guinea pigs with Gp-ic and the transmission of ocular disease to the offspring (3). The present study is concerned with the infection of the male genital tract with Gp-ic and venereal transmission to female guinea pigs.

MATERIALS AND METHODS

The methods for preparation of Gp-ic yolk sac suspension which was used as inoculum, collection of vaginal and conjunctival scrapings, detection of antibodies by indirect immunofluorescence, and histologic preparation of sections have been described previously (3).

Guinea pigs. Mature Hartley strain guinea pigs were obtained from Thorpe Industries, White Bear Lake, Minn., and Simonsen Laboratories, Inc., Gilroy, Calif. All animals were pretested, and only three females from the former source were found to have antibodies to Gp-ic and were excluded from the experiments.

Inoculation and sampling. Male guinea pigs were inoculated intraurethrally with 0.05 ml of Gp-ic material. The penis was extruded from the prepuce by pressing on the lower abdomen. A sterile vinyl tubing (no. 6106, Becton-Dickinson, Rutherford, N.J.) was then inserted about 0.75 inch (1.93 cm) into the urethra. The vinyl tubing was attached to a 23-gauge needle on a 0.25-ml syringe. The inoculation was performed by using an analgesic-tranquilizer mixture (Innovar-Vet, a mixture of fentanyl and droperidol, Pitman-Moore, Inc., Fort Washington, Pa.) to avoid immediate expulsion of the material by urination.

Urethral scrapings of males were collected by use of a dental spatula which could be inserted for a short distance into the urethra. Smears were fixed in methanol and stained with Giemsa.

Detection of antibodies to Gp-ic. BGM cell cultures infected with Gp-ic were prepared in tissue culture chamber slides (no. 4808, Lab-Tek Products Westmont, Ill.) incubated in a flowing 5% CO_2 atmosphere at 37 C with 0.2 ml of medium in each chamber. The slides were collected 3 days after infection when about 40% of the cells showed inclusions. The cells were fixed in cold acetone and stored at −70 C. The silicone rubber spacer was left in place to maintain separation of chamber areas when the reagents were applied and was removed when the preparation was mounted in glycerol.

Detection of Gp-ic in urethral sections by immunofluorescence. The penis was excised and snap-

frozen with dry ice. Frozen sections of 4 μm were cut with a cryostat, air-dried, and then fixed for 3 min in cold acetone. Sections were incubated for 30 min at 37 C with guinea pig antiserum to Gp-ic (titer 320) diluted 1:20. Serum which did not contain detectable antibodies to Gp-ic was used as a negative control. After washing for 30 min in phosphate-buffered saline (PBS), sections were incubated for 30 min at 37 C with rabbit antiserum to guinea pig γ globulin conjugated with fluorescein isothiocyanate (Hyland Laboratories, Costa Mesa, Calif.) diluted 1:20. After another wash in PBS for 30 min, sections were mounted in buffered glycerol. The sections were examined under a Reichert Zetopan microscope and photographed by using Tri-X film (Eastman-Kodak, Rochester, N.Y.).

Skin test. Gp-ic antigen was prepared by infection of BGM cells. The medium was replaced after 2 days, and on day 4 the cells were scraped and frozen at −70 C. After thawing, the material was sonicated for 15 s with a Branson cell disruptor and microtip probe. Titration in embryonated chicken eggs yielded a mean egg lethal dose (ELD_{50}) of log 5.7/0.25 ml. The antigen was heated at 56 C for 20 min which resulted in complete loss of infectivity. Control antigen was derived from uninfected BGM cells.

Skin testing was performed by intradermal injection of 0.2 ml of Gp-ic antigen in one flank and the same volume of control antigen in the opposite flank. The reaction was evaluated by measurement of the diameter of induration at 24 and 48 h. A reaction was considered positive if the diameter of induration was greater than 5 mm at 48 h.

Experimental design. Experiment A. Seven male guinea pigs were each inoculated intraurethrally with approximately 2×10^5 ELD_{50}. Six were caged with from one to three normal females 24 h after inoculation and were removed from the females after 15 days. Urethral smears were made on the males at days 5, 9, and 14. Vaginal smears were obtained from the females at intervals through 23 days.

Experiment B. Nine males were inoculated intraurethrally with approximately 2×10^5 ELD_{50}. They were each caged with two normal females 48 h after inoculation. Vaginal smears were collected from the females at weekly intervals for 10 weeks. Females were removed from males after a period of 23 to 63 days. Urethral specimens were not collected from the males to minimize possible interference with mating and because adequate samples could not be obtained regularly. All animals were skin tested 85 days after the males had been inoculated.

Experiment C. Six males were inoculated intraurethrally with Gp-ic at the same time and dose level as those in experiment B. Four control animals were inoculated with normal yolk sac homogenate. Animals were sacrificed on days 2, 4, and 7. Urethral smears were prepared and the penis was excised. Tissue was collected for histological sections and examination by indirect immunofluoresence.

RESULTS

Response of male guinea pigs to intraurethral inoculation with Gp-ic. (i) Clinical manifestations. Examination of the penis did not reveal significant urethral discharge at any time. Eight of the sixteen males in experiments A and B did, however, manifest single or multiple bullous lesions of the glans and body of the penis (Table 1). The lesions first became apparent 1 to 2 weeks after inoculation and in several animals persisted for about 3 weeks. Microscope examination of smears of lesions from several animals revealed only tissue debris. No further microbiological or histopathological studies were performed.

An unexpected finding was that four of the seven males of experiment A were noted to have a badly mutilated hind leg on day 5 which was attributed at first to the wire mesh cage bottoms. Therefore, in experiment B the animals were placed in cages with solid bottoms which could not catch the legs. Five of the nine animals of experiment B showed the same severe reaction of the right hind leg. In four of them it was observed on the foot on day 8 and resulted in the complete loss of the two lateral toes with retention of the medial toe. The fifth animal was observed to have the same kind of lesion in the hock joint which was first seen on day 15. These lesions healed slowly but all animals survived with closure of the wound. One animal (no. 228) developed unilateral testicular swelling on day 15 and was sacrificed 2 days later. No chlamydial agent was recovered by chicken embryo inoculation of testicular homogenate. No other gross or microscopic pathology was found in this animal.

(ii) Examination of urethral smears. Typical chlamydial cytoplasmic inclusions were observed in cells of day 5 urethral smears from all seven animals of experiment A. Insufficient cells were obtained to make an evaluation of the number of cells infected. There were only moderate numbers of polymorphonuclear cells present. Specimens collected on days 9 and 14 failed to show inclusions. However, on these days, very few specimens were adequate for examination. Among the animals of experiment C, two were found positive for inclusions on day 2 and one on day 4, and smears from the other animals in this experiment were not suitable for examination. The control animals showed no evidence of inclusions or presence of polymorphonuclear cells.

(iii) Histopathology. Sections of urethra of two guinea pigs (no. 227, 236) in experiment C sacrificed on day 2 did not show any inclusions suggestive of Gp-ic infection. Cytoplasmic inclusions were observed in sections from both guinea pigs sacrificed on day 4. Inclusions were limited to the superficial epithelial cells and in

one guinea pig (no. 232) were present in almost all sections (Fig. 1). The demarcation of urethral epithelium from the submucosa was less distinct than in sections from normal animals, and there was an increase in the number of connective tissue cells in the submucosa. Infiltration of polymorphonuclear cells was also present. Inclusions were also detected in all sections of one guinea pig (no. 234) on day 7, whereas none could be observed in another animal (no. 235). Sections from animals inoculated with normal yolk sac homogenate were negative for inclusions and cellular infiltration.

TABLE 1. *Response of male guinea pigs to intraurethral inoculation of Gp-ic*

Expt	Guinea pig no.	Inclusions in urethral smear	Penis lesion	Leg lesion	Antibody titer	Skin test
A	151	+[a]	−	+	≥640[b]	ND[c]
	152	+	+	−	640	ND
	155	+	+	−	160	ND
	156	+	+	−	320	ND
	157	+	+	+	640	ND
	158	+	+	+	≥1,280	ND
	160	+	+	+	≥1,280	ND
B	226	ND	−	−	160	+
	228[d]	ND	−	−	80	ND
	229	ND	−	+	160	+
	230	ND	+	−	80	+
	237	ND	−	+	40	+
	238	ND	−	+	640	+
	239	ND	+	+	640	+
	240	ND	−	−	640	−
	245	ND	−	+	80	+

[a] Symbols: +, positive; −, negative.

[b] Reciprocal of highest dilution yielding positive result as determined by fluorescent antibody method. Sera were collected on day 28.

[c] Not done.

[d] Swollen testicle; sacrificed day 17.

(iv) Immunofluorescence of urethral sections. No definitive staining was observed in sections of urethra from animals inoculated with Gp-ic and sacrificed after 2 and 4 days. Urethral sections from one animal (no. 234) sacrificed after 7 days were clearly positive, whereas material from another animal (no. 235) was weakly positive. Bright cytoplasmic staining was localized in a few superficial cells of the urethra (Fig. 2). No staining was observed with control sera on sections of infected urethra. Sections from animals inoculated with normal yolk sac homogenate were negative.

(v) Antibody response. All 16 males of experiments A and B demonstrated an antibody response to genital tract infection with titers ranging from 40 to ≥1,280 (Table 1).

(vi) Skin test. Positive skin reactions were observed in seven of eight males in experiment B with diameters of induration ranging from 10 to 16 mm. No positive reactions were observed in any animals on the control side.

Transmission of genital infection to female guinea pigs from males infected with Gp-ic. To demonstrate sexual transmission of Gp-ic, inoculated males were caged with uninfected females. A total of 15 males from experiments A and B were housed with a total of 28 females. As indicated by antibody response, all of the males were successfully infected. The females were examined for inclusions in vaginal smears, anti-

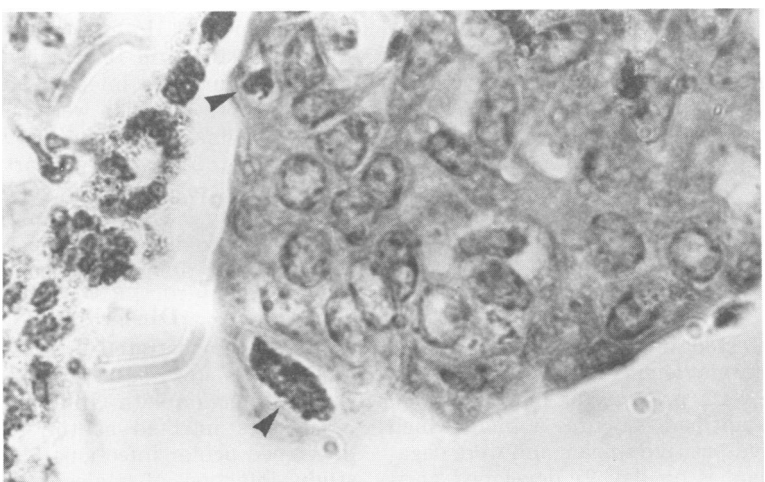

FIG. 1. *Urethral epithelium 4 days postinoculation with Gp-ic (guinea pig no. 232). Two inculsions are present at the epithelial surface (pointers). Giemsa stain. ×630.*

FIG. 2. *Urethral epithelium 7 days postinoculation with Gp-ic (guinea pig no. 234). Bright cytoplasmic staining is present in a superifical cell of the epithelium. Indirect immunofluorescence. ×630.*

body response, development of cellular immunity, and pregnancy. The results are shown in Table 2.

(i) Clinical response. There was no gross clinical response among the infected females, which concurs with our findings reported previously (3). No lesions of the leg joints were recognized in infected females.

(ii) Examination of vaginal smears. Among the 11 females of experiment A, five demonstrated inclusions in cells of vaginal smears. The first day inclusions were found varied from day 8 to day 20.

Seven of the 17 females in experiment B demonstrated inclusions in vaginal smears. The first day, inclusions were found varied from day 5 to day 26.

(iii) Antibody response. Serum was obtained from the females of experiment A after 29 days of caging with males. Antibody was detected in serum of 8 of the 11 animals with titers ranging from 10 to 640. Ten of the 17 females of experiment B which had not demonstrated vaginal inclusions were bled on day 27. One of this group had a titer of 10, whereas the others did not have detectable antibody.

All animals were bled on day 63, and antibody was detected in the serum of 11 of 16 animals with titers ranging from 10 to ≥ 640. All females that were positive for inclusions had demonstrable antibodies. Three animals which had not yielded positive smears and were negative for antibodies on day 27 developed antibodies by day 63.

In experiment A, five of six males transmitted infection to eight females, as evidenced by inclusions in vaginal smears or antibody titers, or both. By the same criteria, in experiment B, seven of nine males transmitted infection to 11 of 16 females. Thus, in our study, a total of 12 of 15 (80%) infected males transmitted Gp-ic infection to a total of 19 of 27 (70%) females.

(iv) Skin test. Five of the 13 females of experiment B gave positive skin reactions with a range of diameter of induration from 6 to 14 mm. No positive reactions were observed on the control side. No positive skin reactions were found among the females without detectable antibody.

(v) Pregnancy. There were no pregnancies in experiment A. In experiment B, eight pregnancies occurred in 16 females. Of these eight animals, four were infected and two of these aborted, both of which were positive for inclusions in vaginal smears and antibody titers. One female (no. 250) was of particular interest. Inclusions were observed in vaginal smears from day 12 to day 26. Smears were then found to be negative up to day 40 and a positive smear was obtained 24 h prior to abortion.

DISCUSSION

It was demonstrated previously that female guinea pigs were susceptible to experimental genital infection with Gp-ic and that offspring of females infected at the time of delivery developed ocular infections (3). In the present study infection of males after intraurethral inoculation was clearly demonstrated. Data included the presence of Gp-ic inclusions in

cells of urethral scrapings and histological sections, detection of Gp-ic antigen in urethral cells by immunofluorescence as well as antibody response, and development of positive skin test. Genital infection of females resulted from caging normal females with infected males. Inclusions in vaginal smears, antibody response, and positive skin tests were observed.

The clinical course of the urethritis was rather mild, but several observations are worthy of note and future experimentation. Many animals developed bullous lesions of the glans and body of the penis. More striking was the development of severe lesions of the right hind legs which led to loss of toes in a number of animals. The pathogenesis of this unexpected response remains to be documented. No marked clinical response was noticed in the female, which was compatible with observations made previously after experimental infection (3). In the course of chlamydial infections of man and animals, lesions of the male genital tract and involvement of the joints are observed. For example, in lymphogranuloma venereum a vesicle on the penis frequently occurs (2) and inflammatory damage to joints is characteristic of polyarthritis of sheep and cattle (5, 6).

Abortion was observed in two of four animals that became pregnant as well as infected. In our former studies on experimental infection of pregnant animals, no abortions occurred (3). This might be accounted for by the fact that they were infected later in pregnancy than those infected by males. However, the number of animals studied permits only speculation at this time, and further experimentation is required to study this important manifestation.

As was reported previously, offspring of experimentally infected females developed ocular infections (3). In the present studies, offspring

TABLE 2. *Response of female guinea pigs caged with males genitally infected with Gp-ic*

Expt	Male no.	Female no.	Inclusions in vaginal smear	Antibody titer			Skin test	Pregnant[a]	Delivery
				Day 29	Day 27	Day 63			
A	151	171	+	40[b]			ND[c]	–	
		172	+	160			ND	–	
	152	159	+	640			ND	–	
		174	–	20			ND	–	
	156	163	–	<10			ND	–	
	157	165	+	10			ND	–	
	158	167	–	<10			ND	–	
		168	+	160			ND	–	
	160	169	–	<10			ND	–	
		175	–	80			ND	–	
		176	–	80			ND	–	
B	226	246	+		ND	≥640	–	+	Abort
		247	–		<10	160	ND	+	Normal
	228	248	–		<10	10	+	–	
		249	–		<10 (died)	ND	ND		
	229	250	+		ND	≥640	+	+	Abort
		251	+		ND	160	+	–	
	230	252	+		ND	80	–	–	
		253	+		ND	160	+	–	
	237	256	–		<10	<10	–	–	
		265	–		<10	<10	ND	+	Normal
	238	257	–		<10	<10	–	+[d]	
		258	–		<10	<10	–	+[d]	
	239	259	–		<10	20	–	–	
	240	261	+		ND	20	–	–	
		262	–		10	160	+	–	
	245	263	–		<10	<10	–	+	Normal
		264	+		ND	80	ND	+	Normal

[a] Experiment A females were caged with males for 15 days. Experiment B females were caged with males for 23 to 63 days.

[b] Reciprocal of highest dilution yielding positive results as determined by fluorescent antibody method. Animals were negative before infection.

[c] Not done.

[d] Found pregnant when sacrificed.

of infected animals did not manifest ocular infection, but the females were apparently infected by males rather early in pregnancy and they were free of infection, as evidenced by absence of inclusions in vaginal smears at the time of delivery. Only one female (no. 250) yielded positive inclusions late after caging, and in this case abortion resulted.

At present, Gp-ic infection is recognized as a mild ocular disease of young animals endemic in many herds. However, the mode of transmission has not been defined and no data are available on genital infection in nature. As an experimental model for the study of genital infection by chlamydial agents, the infection of guinea pigs with Gp-ic appears to be a very promising system and provides the possibility for many different kinds of experimentation.

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