VIROLOGY

Detection of Type 2 Herpes Simplex Virus in Cells of Spermatogenic Epithelium in Infected Testes of Guinea Pigs

S. V. Gribencha, E. E. Bragina*, R. A. Abdumalikov*, E. N. Bocharova, and L. F. Kurilo**

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We developed a model of herpetic orchitis in guinea pigs. Intratesticular inoculation of type 2 herpes simplex virus suspension results in infection of the testicular spermatocytes and spermatides. The possibility of viral infection dissemination from infected into intact testis is proven.

Key Words: *guinea pigs; herpes simplex virus; testicular infection*

Virological studies of human ejaculate are carried out during two recent decades mainly because of the development of *in vitro* fertilization technologies and risk of the oocyte infection. The aim of these studies is, on the one hand, to evaluate the possibility of horizontal transmission of viruses: the blood-testis barrier protecting the viruses in the germinative epithelium from the immune defense and from drug therapy suggests that cells of the ejaculate serve as the infection reservoir. On the other hand, infection of sex cells admits vertical transmission of viruses [10].

Herpes simplex virus (HSV) was for the first time isolated from human ejaculate in cell culture more than 30 years ago. Later HSV DNA was detected by PCR in solitary ejaculate specimens collected between relapses of genital herpes (GH) [14]. Herpes simplex virus was detected in 24-30% ejaculate specimens from patients with a history of GH and fertility disorders [1,2,6]. The low level of PCR-detection of HSV in the ejaculate of patients without symptoms of GH can be explained by specific features of the semen as the substrate for such a study [11]. The virus was detected by modified PCR method in spermatozoa of 50% infertile patients without GH symptoms [7].

Virological study of human ejaculate is important in artificial fertilization and sterility. However, the problems of virus location and mechanism of HSV infection of the semen are solved at the hypothetical level.

HSV is a nuclear virus; its DNA replicates in the cell nucleus and the nucleus-cytoplasm transport of HSV RNA molecules and various (regulatory and structural) proteins is an obligatory component of normal cycle of virus development. It is hardly possible that HSV cycle is realized in mature spermatozoa, characterized by chromatin condensation, absence of pores in the nuclear membrane, and absence of the nucleus-cytoplasm transport. Presumably, HSV cycle is realized in immature sex cells during spermiogenesis. However, the data on the possibility of HSV development in immature

D. I. Ivanovskii Institute of Virology, Russian Academy of Medical Sciences; *A. N. Belozerskii Institute of Physicochemical Biology, M. V. Lomonosov Moscow State University; **Medical Genetic Research Center, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** belnick@mail.ru. E. N. Bocharova

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sex cells are contradictory. It was reported that herpes viruses develop in Leydig's, but not in spermatogenesis cells [13]. On the other hand, it was shown that immature sex cells can be infected with HSV [5]. Type 2 HSV was found in the testes of 4 of 10 corpses in autopsy, this suggesting that this organ served as a reservoir of the infection in virus transmission. Study of the relationship between male infertility and presence of HSV or adenovirus in the ejaculate or testes showed that this or that virus was present in 40% infertile patients [5]. Inoculation of HSV into mouse testes led to infection of testicular tissue, which was confirmed by virus isolation in cell culture [8].

We developed a model of herpetic orchitis in guinea pigs by direct infection of the testes. Infection of immature sex cells (spermatocytes and spermatides) was observed after intratesticular inoculation of HSV2 mixture.

MATERIALS AND METHODS

Strain mS-5, a kind gift from Dr. N. N. Mel'nikova (Institute of Viral Preparations), was used in the study. The HSV2 infective material was obtained by 5 successive passages of mS-5 strain after infection of Vero cell monolayer with the virus in 1:1000 dilution. The virus titer during passage 5 was 5.2 lg TPD_{50}/0.1 ml. Virus specificity was confirmed in cross neutralization test in cell culture (virus neutralization titer >2 lg TPD_{50}/0.1 ml) and in indirect immunofluorescence test with monoclonal antibodies to superearly proteins of HSV2 (Sanofi).

A new method for infection of guinea pig testes is developed: the abdominal cavity is not opened and the contralateral testis remains intact and serves as the control.

In order to verify the accuracy of injection and the method of testicular infection visually, 2% methylene blue solution was injected into the testis (20-30 μl). Opening of the abdominal cavity after 40-60 min showed dark-blue testis, into which methylene blue was injected, in contrast to light-pink intact testis.

The testes were infected with HSV2 by injection of the virus (20-25 μl) into the left testis in 12 male guinea pigs. The animals were injected intratesticularly in the following doses: group 1) 500 lg TPD_{50}/0.02 ml; 2) 5000 lg TPD_{50}/0.02 ml; 3) 25,000 lg TPD_{50}/0.02 ml. Controls were injected with 20-25 μl isotonic NaCl solution.

The HSV antigen (AG) was detected in testicular cells by direct immunofluorescence with polyclonal antibodies to HSV (Institute of Influenza; lot 162, working dilution 1:16) and with monoclonal antibodies to immediate early HSV2 proteins (Sanofi).

Impression smears were prepared routinely, fixed in cold acetone for 10 min, incubated with luminescent serum for 30 min at 37°C, washed in buffer (20 min) and distilled water (20 min), dried, and examined under a LUMAM-2 fluorescent microscope under water immersion.

TABLE 1. Dose and Duration of Intratesticular Infection of Guinea Pigs with HSV2 Suspension

Animal No.	Infective dose	Day	Left testis			Right testis		
			size, mm	focus	HSV AG	size, mm	focus	HSV AG
1	500 lg TPD_{50}	2	19×8	+	3-4	19×15	—	0
2	500 lg TPD_{50}	4	18×8	—	3-4	20×15	—	0
3	500 lg TPD_{50}	7	17×8	+	4	19×15	—	2
4	500 lg TPD_{50}	7	17×9	—	0	18×10	—	0
5	5000 lg TPD_{50}	2	18×10	+	4	18×14	—	0
6	5000 lg TPD_{50}	4	18×7	+	3	20×15	—	0
7*	5000 lg TPD_{50}	N.d.**	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.
8	5000 lg TPD_{50}	7	17×8	+	4	19×15	—	0
9	25,000 lg TPD_{50}	2	19×9	+	4	19×15	—	2-3
10	25,000 lg TPD_{50}	4	21×10	Necrotic foci	4	21×14	—	2-3
11***	25,000 lg TPD_{50}	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.
12	25,000 lg TPD_{50}	7	18×14	+	4	20×15	—	0
13	Control	2	18×14	—	0	19×15	—	0
14	Control	7	18×15	—	0	19×14	—	0

Note. *animal died 3 days after infection; **not determined; ***inadequate behavior was observed 4 days after infection.

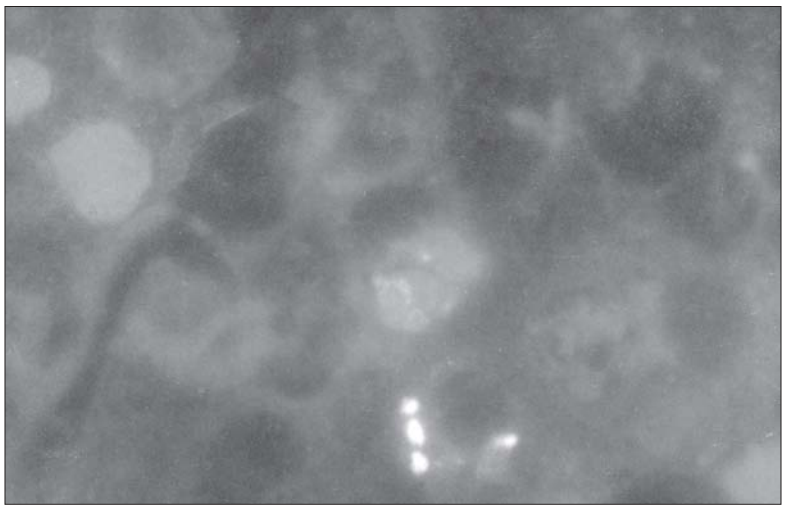

Fig. 1. Impression smear of guinea pig testicular cells 4 days after infection with HSV2 suspension in a dose of 25,000 lg TPD_{50}/0.02 ml. Infected cell containing solitary granules of HSV AG in the group of spermatocytes tightly adhering to each other. Here and in Fig. 2: immunofluorescence test with polyclonal antibodies to HSV, ×600.

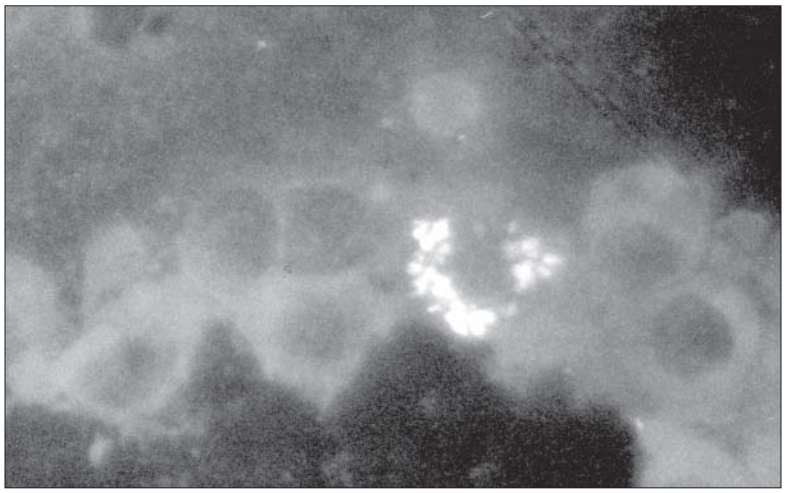

Fig. 2. Impression smear of guinea pig testicular cells 7 days after infection with HSV2 suspension in a dose of 5000 lg TPD_{50}/0.02 ml. Infected spermatocyte contains numerous HSV AG granules.

RESULTS

Twelve guinea pigs were infected with HSV suspension; 2 animals were excluded from the study. Pronounced enlargement of the infected testes, hyperemia, and enhanced vascular pattern were observed starting from day 2 postinfection. Foci of infection presented as yellowish infiltration, herpetic bubbles, or, in some cases, as necrotic foci (Table 1). The right intact testes were unchanged and pink-colored. Minor hyperemia of the left testes was observed in controls 4 and 7 days after injection of saline. The size of the testes did not change after inoculation.

The results of direct immunofluorescence test were positive for the infected left testes in 9 of 10 animals. HSV AG in infected testicular impression smears was visualized as granules in the nucleus, perinuclear zone, and cytoplasm of spermatocytes and round spermatides (Fig. 1). Multiplicity of infection increased by day 7 (Fig. 2). No HSV AG was detected in impression smears from control animals.

Granules of HSV AG were detected only in the infected testis in the majority of animals, while the contralateral testis contained no viral proteins. Solitary incorporation of viral AG were detected in the intact testis of one animal after infection dose of 500 lg TPD_{50}/0.02 ml 7 days after HSV inoculation and in two animals after infective dose of 25,000 lg TPD_{50}/0.02 ml 2 and 4 days after the virus inoculation (Table 1).

Spermatozoa with heads containing HSV AG were detected in impression smears of the infected testis of one animal infected with HSV suspension in a concentration of 25,000 lg TPD_{50}/0.02 ml.

A model of infection of mouse testes was suggested previously [8]: the testes were infected with mouse cytomegalovirus or HSV under conditions of opened abdominal cavity or with opening of the scrotum and subsequent suturing. We developed a new method for direct infection of guinea pig testes without opening the scrotum. Injection of methylene blue solution showed that the contralateral testis remained intact.

An important aspect of the testicular sex cell infection is the location of infection in asymptomatic GH. Great attention is paid to this problem in recent years, because asymptomatic release of the virus is the main cause of high prevalence of GH in many population groups [3,12]. The problem of asymptomatic HSV infection of the male genital tract is less studied than asymptomatic carriership in women.

Infection of the male gametes by incubation of mature spermatozoa with a suspension of infective virus revealed virus adhesion to the cell surface [4,9]. The authors failed to detect the infection inside the gametes and concluded that the viral particles could be eliminated from the surface of mature spermatozoa.

We detected the fact of HSV2 replication in spermatogenic epithelial cells of experimental animals. Hence, a heretofore not described model of herpetic orchitis, induced in guinea pigs by direct infection of the testes, was developed.

Detection of HSV2 AG in the spermatozoon heads on impression smears indicates that the intragamete infection of the spermatozoa is presumably realized at the level of spermatogenic epithelium.

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