Experimental *Mycoplasma hominis* Infection of the Genital Tract in BALB/c Mice

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 1, pp. 62-65, January, 2004
Original article submitted July 8, 2003

We studied the ability of laboratory and clinical strains of *Mycoplasma hominis* to colonize the genital tract mucosa in BALB/c mice. Colonization with mycoplasma occurred only in mice receiving estrogen. *Mycoplasma hominis* strains obtained after 3-fold passage through the vaginal mucosa in mice and administered intravaginally in a dose of 0.5×10^8 CFU/ml caused infection in 100% animals. Inflammation in the lower genital tract was reduced by the 8th week after infection.

Key Words: *Mycoplasma hominis; experimental infection*

Mycoplasma hominis is a mycoplasma species isolated from humans and belonging together with *M. genitalium*, and *M. urealiticum* to the group of urogenital microorganisms. Widespread distribution of urogenital mycoplasmas and their presence in clinically healthy people cast some doubt on their role in the pathogenesis of urogenital diseases. Some authors classify mycoplasmas to absolute pathogens, while others believe that they are opportunistic microorganisms [1,2, 6,7]. Pathogenicity and virulence are estimated on various models of infection with different clinical strains of microorganisms. Experimental infection allows evaluating *in vivo* susceptibility of microorganisms to antibiotics. This method is extensively used in the search for new drugs and therapeutic schemes. It is of considerable importance with regard to mycoplasma infections, since high genomic variability of mycoplasmas determines appearance of new resistant strains, while specific features of metabolism allow mycoplasmas to avoid immune reactions in the host macroorganism [3,5]. Virulence and antibiotic sensitivity of mycoplasma strains are *in vivo* studied on susceptible laboratory animals. However, new preparations should be tested on mycoplasma strains that cause a reproducible infectious process.

Here we compared the abilities of laboratory and clinical strains of *M. hominis* to colonize the genital tract in BALB/c mice, described experimental infection, and selected the strain with most pronounced virulent properties.

MATERIALS AND METHODS

Experiments were performed on female BALB/c mice aging 6-8 weeks and weighing 18-22 g. Clinical strains of *M. hominis* were isolated from patients with nonspecific inflammatory diseases of the urogenital tract and presented by A. E. Taraskina (D. O. Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences, St. Petersburg). Strain 1862.3 carried the tetracycline resistance determinant tetM; strain 574 did not carry tetM. Laboratory strain H-34 was presented by I. V. Rakovskaya (N. F. Gamaleya Institute of Epidemiology and Microbiology). Mycoplasma strains were cultured on Brain Heart Infusion medium (DIFCO) containing 10% yeast extract, 15% horse plasma (Biolot), 10 g/liter arginine, 600,000 U/liter penicillin, and 1.2 ml/liter 5% thallium acetate.

Hexestrol in a dose of 0.5 mg/kg (Farmadon, 0.05 ml) was injected subcutaneously to synchronize the estrous cycle. Treatment was repeated 4 times at 1-week

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0007-4888/04/1371-0053

intervals. The mycoplasma suspension (50 μl) was administered intravaginally using an Eppendorf automatic pipette immediately after the second injection of hexestrol. Mycoplasmas were grown in a liquid nutrient medium, concentrated by centrifugation, and washed 3 times with buffered physiological saline (pH 7.4). Control animals received sterile physiological saline.

We compared virulence of mycoplasma strains. The dose of mycoplasma strains causing infection in 50% animals (ID_{50}) was estimated by the Keber formula. The range of infecting doses was 0.5×10^6-0.5×10^{11} CFU/ml. Each group included 6 animals.

In further experiments we determined the time of persistence and titer of mycoplasmas in the lower (vaginal mucosa) and upper genital tract (aseptically isolated ovaries, fallopian tubes, and upper third of the uterine horn). Samples were taken weekly for 9 weeks starting from the 1st week after infection. We examined 20 mice. Epithelial cells were scraped from the vaginal mucosa using disposable sterile probes (DNC Med) and placed in 1 ml liquid nutrient medium. Two mice were killed once a week to obtain homogenates of organs. Tenfold serial dilutions of the primary sample (final dilution $1:10^{10}$) in 0.5 ml medium with arginine and phenol red (indicator) were prepared to estimate the titer of *M. hominis*. Dilutions were incubated at 37°C not less than for 10 days. Maximum dilution of the inoculum causing mycoplasma growth and changing color of the indicator was taken as *M. hominis* titer and expressed in CCU/ml (color-changing unit).

The vaginas of treated and control mice were fixed with 10% neutral formalin and embedded into paraffin. The sections (5 μ) were stained with hematoxylin and eosin and examined under a light microscope (Biolam).

The results were analyzed by Student's *t* test (Microsoft Excel 2000).

RESULTS

Mycoplasma strains administered intravaginally in various infecting doses did not colonize the genital tract in mice not pretreated with the hormone. Samples of the vaginal mucosa on day 6 after infection were mycoplasma-negative.

M. hominis colonized the vaginal mucosa in animals pretreated with the hormone. Interstrain differences were revealed in the severity of inflammation and period over which the infectious agent could be isolated. ID_{50} for *M. hominis* strains H-34, 1862.3, and 574 were 0.5×10^{10}, 0.2×10^6, and 0.48×10^7 CFU/ml, respectively. These differences in the colonization ability are related to controlled regulation of the main pathogenisity factors under various environmental conditions. Mycoplasmas have a labile system for activation of protein adhesins [4,5]. After long-term culturing on synthetic nutrient media they lose adhesive properties necessary for colonization of the mucosa. Contact with the host organism induces synthesis of adaptive factors by the microorganisms. The next series was performed with clinical strains of *M. hominis*. Mycoplasma strains were 3-fold passed through the vaginal mucosa in BALB/c mice to enhance adhesive activity. Colonization potential of mycoplasmas increased after this treatment. The time of persistence for strain 1862.3 increased 7-fold to the control (7 and 1 weeks, respectively). The time of persistence for strain 574 increased from 3 to 6 weeks (by 2 times). It should be emphasized that mycoplasmas were isolated from 100% mice over the first 2 weeks after infection (Table 1).

Intravaginally administered mycoplasma caused infection of the lower genital tract. Samples of the upper genital tract (ovaries, fallopian tubes, and upper third of the uterine horn) were micoplasma-negative. No differences were revealed between the dynamics of *M. hominis* persistence.

Microscopic examination of histological sections revealed signs of acute inflammation in the vaginal mucosa (agglomerates of leukocytes) and submucosa (infiltration with leukocytes) in mice discharging mycoplasma (Fig. 1). Primary infection resulted in elimination of mycoplasmas and reduction of acute inflammation did not protect these mice from reinfection. It should be emphasized that during reinfection the infectious agent could be isolated for a shorter period.

Our results show that mycoplasma colonization of the genital tract occurs only in BALB/c mice receiving estrogen. Previous studies showed that administration

TABLE 1. Isolation of *M. hominis* from the Vagina of BALB/c Mice (*M*±*m*, lg CCU/ml)

Period, weeks	*M. hominis* strain	
	574 (third passage)	1862.3 (third passage)
1	7.875±1.100	8.25±0.75
2	5.875±1.300	6.375±1.170
3	3.50±0.94	4.85±1.10
4	2.375±1.140	4.66±1.33
5	1.125±1.120	7.2±1.8
6	0	3.00±1.89
7	0	1.8±1.8
8	0	0

Note. Infecting dose 0.5×10^8 CFU/ml.

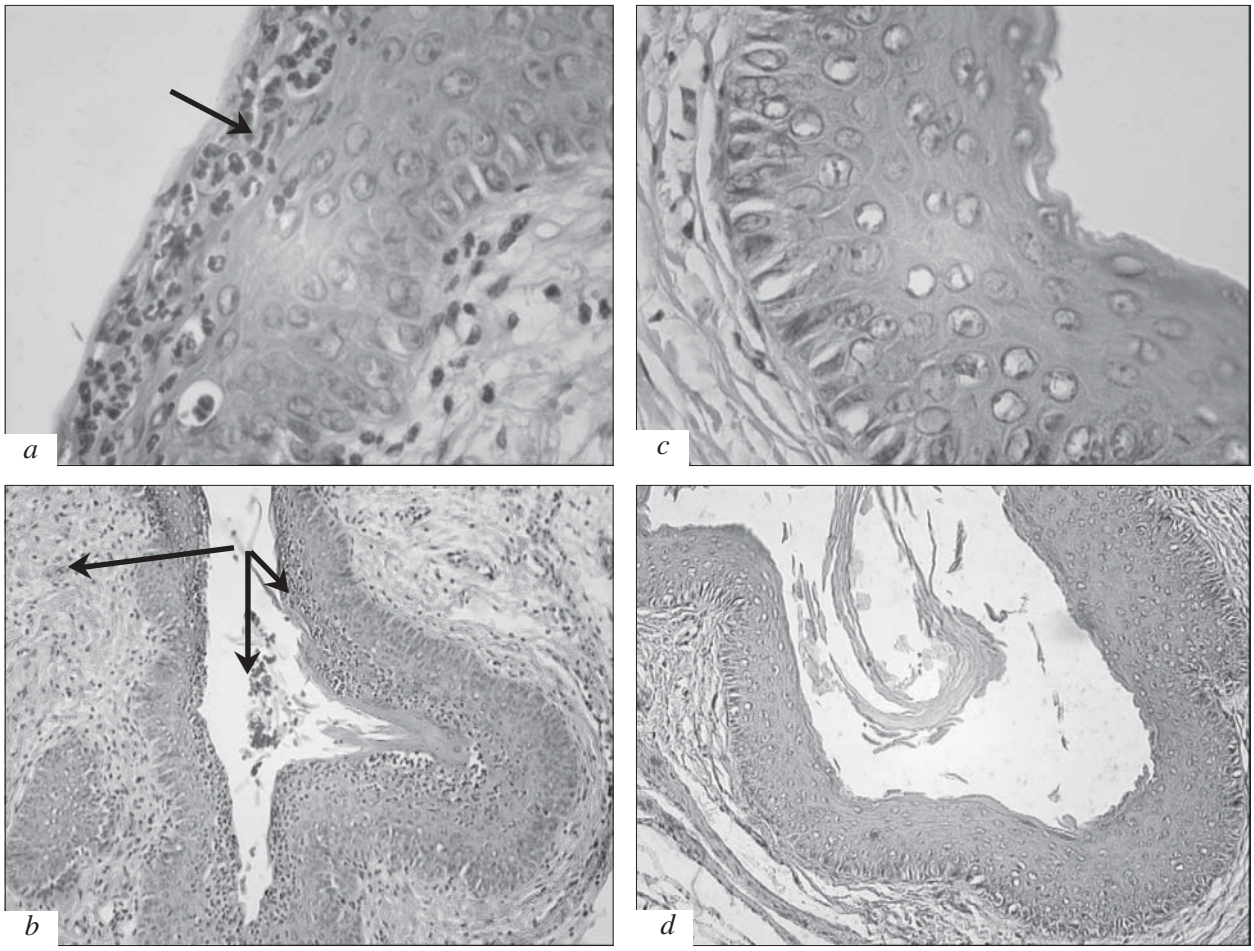

Fig. 1. Histological sections of the vagina from BALB/c mice on day 28 after infection with *M. hominis* strain 1862.3 (*a*, *b*) or administration of physiological saline (*c*, *d*). Arrows: agglomerates of leukocytes. ×240 (*a*, *c*); ×120 (*b*, *d*).

of the estrogen to adult female mice breaks the estrous cycle, stimulates growth and differentiation of the multilayered squamous epithelium in the vaginal mucosa, and initiates glycogen synthesis in epithelial cells and mucus production in endocervical glands. It promotes survival and reproduction of estrogen-dependent *M. hominis* [8]. Colonization potential was similar in strain 1862.3 carrying the tetracycline resistance determinant tetM and strain 574 not carrying this determinant. It should be emphasized that passage of strain 1862.3 through the animal organism increased the doxycycline sensitivity threshold. Threefold passage of *M. hominis* through the vaginal mucosa in BALB/c mice allowed us to isolate strains that reproducibly caused genital mycoplasmosis.

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