PRIMATOLOGY

Experimental Mycoplasma Infection in Monkeys

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Experimental mycoplasma infection was studied in *Papio hamadryas, Macaca mulatta,* and *Macaca nemestrina* infected with Mycoplasma (*M. pneumoniae* and *M. hominis*) and Ureaplasma (*U. urealyticum*).

Key Words: *experimental mycoplasma infection; respiratory tract; urogenital tract; monkeys*

Various aspects of human mycoplasma infections were studied on laboratory animals. Monkeys (particularly, chimpanzee) have been acknowledged as the best laboratory animals for studying this human infection [5]. Respiratory mycoplasma infection induced by *M. pneumoniae* [2], arthritis induced by *M. hominis, M. arthritidis,* and *U. urealyticum* [3], urethritis in males infected with *U. urealyticum* [12], and *M. genitalium* [13,14] were studied on chimpanzee.

The efficiency of modeling human mycoplasmoses in monkeys [1,4-12,14,15] depends on the strain used for infection and monkey species. This was taken into consideration in experimental study of mycoplasma infection monkeys of the Sukhumi and Adler Centers. These experiments were carried out in collaboration with N. F. Gamaleya Institute of Epidemiology and Microbiology (Moscow) and Institute of Veterinary Medicine, Academy of Sciences of Hungary (Budapest).

MATERIALS AND METHODS

The study was carried out on 30 male monkeys: 20 *M. mulatta,* 7 *M. nemestrina*, and 3 *P. Hamadryas*. Seven animals (4 *M. mulatta,* 2 *M. nemestrina*, and 1 *P. hamadryas*) served as controls. All animals were kept in individual cages and had no antibodies to Mycoplasma or Ureaplasma before the experiment. Five days before infection the monkeys were orally treated with tetracycline in a dose of 0.1 mg/kg. Inoculated and control animals were thoroughly examined. The examination included clinical, cytological, X-ray, microbiological (reisolation of the agents at various terms of experiment from different materials), and serological (detection of antibodies) studies. Some animals were examined pathomorphologically. All manipulations with infection and sacrifice were carried out under calypsol anesthesia. Organ and tissue fragments for morphological study were fixed in 10% neutral formalin, paraffin sections were stained with hematoxylin and eosin.

For modeling of respiratory mycoplasmosis we used a 24-h broth culture of *M. pneumoniae* (10^7 CFU/ml) fresh-isolated from a child with pneumonia. Isolation and culturing were carried out in medium B [6]. Six *M. mulatta* aged 1-6 years received 1 ml culture through an intubation tube inserted into the trachea. One monkey was sacrificed on day 9 (at the peak of clinical manifestations), 2 on day 15, 2 experimental and 1 control (received 1 ml sterile culture medium intratracheally) monkeys on day 65.

Urogenital mycoplasmosis was modeled in 8 *M. mulatta* (with 2 controls) aged 6-12 years, 7 *M. nemestrina* aged 6-8 years (2 controls), and 3 *P.*

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hamadryas aged 8-12 years (1 control). *M. hominis* was cultured in medium B [6], *U. urealyticum* in medium prepared according to the D recipe [12]. *M. hominis* and *U. urealyticum* were isolated from the blood and genital tract of women with pathological labor. The monkeys were infected intraurethrally with a rubber catheter inserted to a depth of 3-5 cm. Five monkeys (3 *M. nemestrina* and 2 *M. mulatta*) received 0.5 ml of 24-h serovar II *U. urealyticum* monoculture (10^6 color-forming units — CFU); 2 *M. mulatta* were infected with 48-h broth culture of *M. hominis* (1.3×10^7 CFU/ml, 1 ml), 2 *M. nemestrina,* 2 *M. mulatta*, and 2 *P. hamadryas* were infected with a mixture of equal volumes (0.5 ml) of these cultures. Controls received the same volumes of sterile culture media intraurethrally.

After 10 days both *M. mulatta* infected with *M. hominis*, one *M. mulatta* infected with *U. urealyticum*, and all *P. hamadryas* (1 control and 2 experimental) and one *M. mulatta* infected with a mixture of Mycoplasma and Ureaplasma were sacrificed. After 30 days 1 *M. nemestrina* infected with Ureaplasma, 1 *M. nemestrina* infected with a mixture of Mycoplasma and Ureaplasma, and 1 control *M. nemestrina* were sacrificed. Two *M. nemestrina* inoculated with Ureaplasma and 1 *M. nemestrina* inoculated with a mixture of Mycoplasma and Ureaplasma were reinfected after 35 days ac-

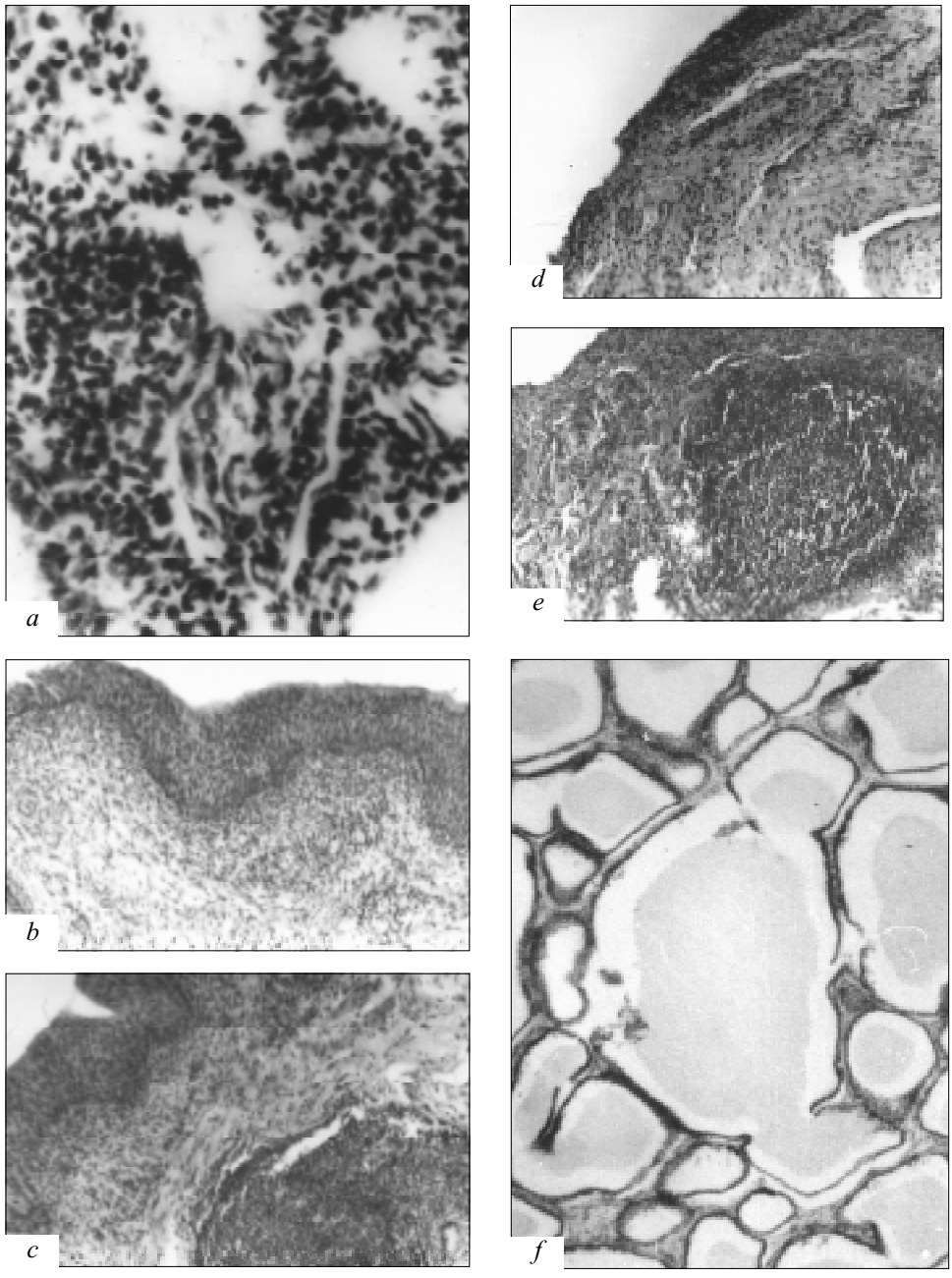

Fig. 1. Experimental mycoplasma infection in macaques. Hematoxylin and eosin staining, ×120 (*a*), ×40 (*b-f*). *a*) interstitial pneumonia after infection with *M. pneumoniae* (*M. mulatta* No. 21,608); *b*) cystitis after infection with *U. urealyticum* (*M. nemestrina* No. 15,357); *c*) cystitis induced by *M. hominis* and *U. urealyticum* mixture (*M. nemestrina* No. 15,871); *d*) erosion in the urethra after infection of *M. hominis* and *U. urealyticum* mixture (*M. nemestrina* No. 15,871); *e*) congestion of prostatic secretion after infection with *M. hominis* (*M. mulatta* No. 366); *f*) erosive cystitis with the formation of lymphatic folliculus (*M. mulatta* No. 17,884).

cording to the same protocol and sacrificed on days 11, 27, and 40 after the second inoculation (on days 46, 62, and 75 after the first inoculation). On day 40 (day 75 of experiment) the other control *M. nemestrina* was sacrificed.

The duration of Ureaplasma persistence in the organism, location of the agents, and types of morphological reactions in tissues under conditions of persisting agent were studied in *M. mulatta*. Four *M. mulatta* (4-6 kg, aged 3-5 years) were infected intraperitoneally in a single dose (5×107 CFU/ml) with 24-h *U. urealyticum* (serovar VIII) culture and sacrificed 3, 6, 12 weeks and 6 months postinfection. Morphological changes in tissues and organs were correlated to the presence of Ureaplasma antigen detected by indirect immunofluorescent test (IIFT) with rabbit hyperimmune serum to serovar VII *U. urealyticum,* FITC-labeled antirabbit globulin, and rhodamine-labeled bovine globulin for the background contrasting (manufactured by N. F. Gamaleya Institute of Epidemiology and Microbiology). The test was carried out on tissue impressions fixed in 98% ethanol. Ureaplasma cultures isolated from monkey tissues were identified in the metabolism inhibition test using rabbit immune serum to serovar VIII *U. urealyticum*.

RESULTS

The organs were sterile in all control animals, no clinical signs of Mycoplasma infection or pathomorphological changes were detected.

Weakness, dullness, hyperemia of the posterior laryngeal wall, harsh breathing and rales in the lungs, dyspnea, increased erythrocyte sedimentation rate (5-12 mm/h *vs.* 2-4 mm/h normally), and neutrophilic leukocytosis were observed in 5 of 6 *M. mulatta* infected with *M. pneumoniae*. Mycoplasma could be reisolated from the throat on days 4-35 and from the blood on days 9-25 postinoculation. Morphological analysis showed interstitial pneumonia (Fig. 1, *a*) and lymphoid infiltration in the submaxillary salivary gland (monkey No. 21,608), tonsillitis (monkey No. 21,176), bronchitis (monkey No. 21,147), cystitis (monkey No. 95), foci of lymphoid infiltration in the lungs and kidneys (monkey No. 75), and lymphoid hyperplasia (LH) in the lungs (in 4 monkeys), spleen, and lymph nodes (in 1 monkey). Bacteriological study showed that Mycoplasma can be isolated from the lungs, trachea and bronchi, tonsils, spleen, and kidneys in all cases; almost always (in 4 of 5 monkeys) from the urinary bladder and submaxillary salivary gland; less often (in 3 monkeys) from the seminal vesicles, pancreas, and peripheral lymph nodes; and rarely (in 2 monkeys) from the heart, testicles, thyroid, and urethra. Serological studies showed antibodies in 1:32 titer only in monkey No. 95 on day 28 postinoculation; by day 65 they disappeared.

Hence, a model of respiratory mycoplasmosis was obtained on *M. mulatta*. The disease was characterized by involvement of the respiratory and urogenital organs, pronounced immunomorphological reaction in the lungs, rarely in the spleen and lymph nodes, and generalization and long persistence (up to 65 days) of the agent in the organs.

Urethral mycoplasmosis was induced in 3 monkey species infected with *M. hominis* and *U. urealyticum* (monocultures and their combination).

Three days after infection with Mycoplasma and Ureaplasma mixture and monocultures, frequent intermittent urination, serous exudation from the urethra, and edema at the urethral opening were observed in all monkeys, their clinical condition was satisfactory; after reinfection these symptoms were appeared earlier (on day 1). Numerous desquamated epithelial cells and segmented leukocytes were detected in urethral smears. These changes were more pronounced in *M. nemestrina* infected with mixed cultures; these symptoms persisted until day 17 after the first inoculation and until day 10 after the second inoculation. In *M. mulatta* the changes were observed only until day 5 and were less pronounced after infection with *M. hominis* and in monkey No. 19,436 infected with mixed culture. Ureaplasma was reisolated from the urethra of *M. nemestrina* on days 4-24 after the first inoculation with monoculture and on days 4-10 after the second inoculation. After inoculation with mixed culture, Mycoplasma was isolated until day 10 and Ureaplasma until day 24 after the only inoculation and the first of the two inoculations, respectively. After the second inoculation, both Mycoplasma and Ureaplasma were isolated from the urethra on days 4-11. In *M. mulatta* Ureaplasma were reisolated from the urethra on days 3-13 and 3-20 (after inoculation with monoculture and mixed culture, respectively) and from the blood on days 7-13 and 7-20, respectively. The period of *M. hominis* reisolation from the urethra and blood of *M. mulatta* infected with mono- and mixed culture were often shorter than for Ureaplasma.

Ureaplasma could not be reisolated from the organs of 2 of 5 sacrificed *M. nemestrina*; no morphological changes were seen in these animals. In 3 other monkeys Ureaplasma or both agents were isolated from the urethra and urinary bladder on days 40 and 11 after repeated inoculation and on day 30 after a single inoculation with mixed culture. Changes in the urinary bladder and urethra (cystitis, urethritis) were detected in all these animals (Fig. 1, *b-d*).

In 4 *M. mulatta* sacrificed on day 10 postinoculation the agents were detected in the urine, urethra. urinary bladder mucosa, testicle, prostate, spleen, rarely in the liver and kidneys. Infection with *M. hominis* monoculture led to LH in the spleen (and the agent was reisolated from this organ) in 1 monkey. In another monkey congestion with formation of cysts in the prostate was observed (Fig. 1, *e*), with suppressed spermatogenesis in the testes (it is noteworthy that Mycoplasma was isolated from these organs). *M. mulatta* infected with Ureaplasma developed urethritis and cystitis (Fig. 1, *f*) and sialadenitis of the submaxillary salivary gland, interstitial pneumonia and LH in the spleen. Urethritis, cystitis, prostatitis, sialadenitis, interstitial hepatitis, LH in the spleen, visceral lymph nodes, and lymphoid formations of the intestine, paralleled by generalization of infection, were detected in *M. mulatta* infected with a mixture of Ureaplasma and Mycoplasma.

P. hamadryas had clinically asymptomatic infection even after inoculation with a mixed culture. Only Ureaplasma was reisolated on days 3-7 postinoculation. Only Ureaplasma was reisolated from the urethra and urinary bladder in postmortem examination on day 10 postinoculation. Morphological study showed pathological processes in the urinary tract (cystitis and urethritis) (Fig. 2, *a*, *b*) and suppressed spermatogenesis in the testes.

Hence, the pathogenicity of *M. hominis* and *U. urealyticum* for three monkey species was confirmed. The disease does not make the animals resistant to reinfection, after which the symptoms manifest even earlier than after the first infection, but sooner disappear. Generalization of infection was demonstrated: the agents were reisolated not only from the urethra and urinary bladder, but from the testes, prostate, and other organs, in which morphological changes were detected (urethritis, cystitis, prostatitis, lymphoid hyperplasia in the spleen and lymph nodes, sialadenitis). Generalization was more pronounced in *M. mulatta*, in which the agent was detected in the blood throughout life. Ureaplasma persisted for up to 40 and Mycoplasma up to 30 days postinfection. Morphological signs of Mycoplasma tropism to

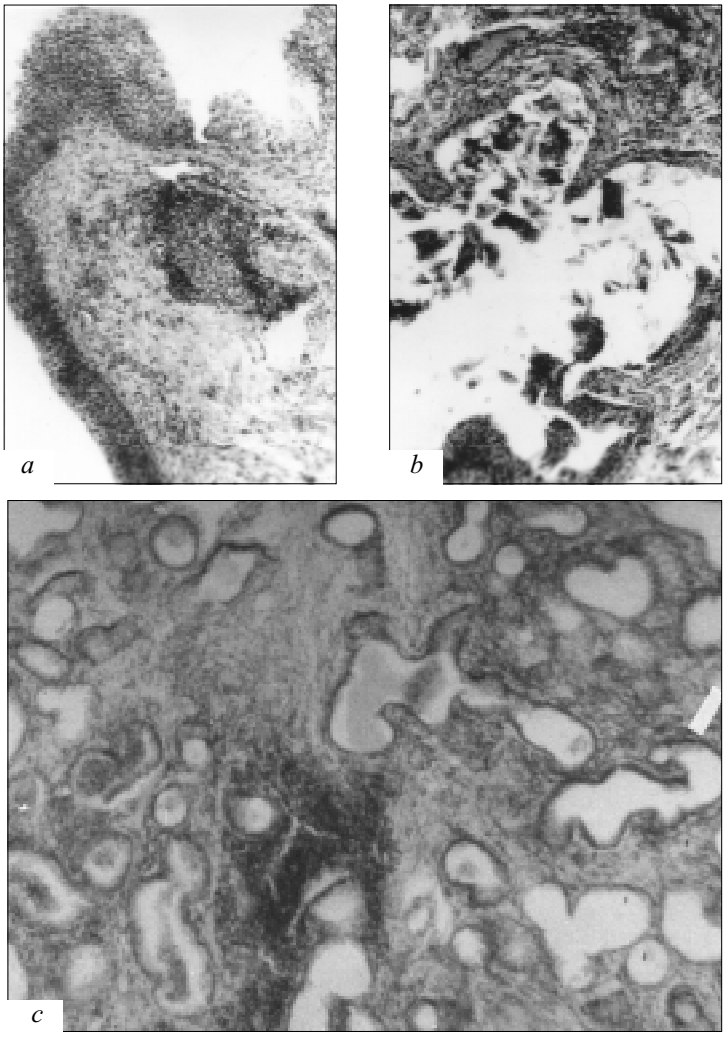

Fig. 2. Urogenital mycoplasma infection in *P. hamadryas* and *M. mulatta*. Hematoxylin and eosin staining, ×40. *a*) cystitis after infection with a mixture of Mycoplasma and Ureaplasma (*P. hamadryas* No. 17,225); *b*) urethritis after infection with a mixture of Mycoplasma and Ureaplasma (*P. hamadryas* No. 15,841); *c*) prostatitis after infection with Ureaplasma (*M. mulatta* No. 20,899).

immunogenesis organs were observed in early periods postinoculation. The agents were detected in these organs, and morphologically expressed hyperplastic reaction was observed).

U. urealyticum in titers 10^3-10^4 CFU were isolated 24 h postinoculation from the blood of *M. mulatta* intraperitoneally infected with serovar VIII *U. urealyticum* and were detected throughout the entire 6-month period of observation. Ureaplasma in smears from the throat were isolated only from 2 monkeys starting from days 3-7 postinoculation throughout the entire period of observation (21 and 42 days). Analysis of the organs, urine, and bile 3 weeks postinoculation showed generalization of the agent, which could be reisolated or detected by IIFT from almost all examined material, except the bone marrow, inguinal lymph nodes, thyroid, prostate, urethra, brain and spine. 6-12 weeks postinoculation the agent could be reisolated only from some materials: Ureaplasma were isolated from the liver and spleen of both monkeys, from mesenteric lymph node and thymus of one monkey, and from the liver and adrenal of the other one. On the other hand, IIFT showed the presence of the agent in 8-9 organs besides the organs from which it was reisolated, which indicates much greater generalization of the agent. Six months postinfection Ureaplasma were isolated only from the blood and liver, while IIFT showed the antigen not only in the liver, but in the bone marrow, kidney, spleen, lymph nodes, testicle, pituitary, thymus, urinary bladder, and spine.

Morphological study showed LH in the spleen, abdominal lymph nodes, and intestine in all monkeys. This reaction was the least expressed in monkey No. 20,193, which had no other changes. Monkey No. 20,900 had also lymphocyte infiltration of the pleura and interalveolar septa in a small area of the lower lobe of the right lung (near the site of Ureaplasma inoculation) and cystitis. Monkey No. 20,860 had prostatitis with formation of lymphatic folliculi, monkey No. 20,899 prostatitis (Fig. 2, *c*), hepatitis, interstitial pneumonia, and bronchitis.

Hence, a single intraperitoneal infection with Ureaplasma led to their dissemination in the body, long persistence, and reproduction during 6 months of observation, which was confirmed by reisolation of the agent from the blood. Accumulation of Ureaplasma and its antigens in immunogenesis organs (spleen, lymph nodes, bone marrow, thymus, and intestine) and in the urogenital system confirmed their tropism to these tissues, which was observed in other variants of inoculation and virtually in all variants of mycoplasma infection. The presence of Ureaplasma in these tissues can be paralleled by pathological processes.

Hence, lower monkeys are sensitive to infection with *Mollicutes* representatives (*M. pneumoniae*, *M. hominis*, and *U. urealyticum*) isolated from humans. The disease involved changes similar to those observed in humans, which indicates that monkeys are perspective laboratory animals for studies of these human infections. Common characteristics of the studied Mycoplasma were detected: capacity to activate hyperplastic processes in immunogenesis organs, induce lesions of lung tissue and urogenital mucosa, persist in the body for a long time without inducing any changes. This latter characteristic explains high prevalence of the infection in monkeys. The persistence in healthy monkeys is often asymptomatic and confined to the mucosa. Activation of infection with clinical morphological changes develops under conditions of decreased immunological reactivity. Mycoplasma are then detected in internal tissues and show tropism to immunogenesis organs, lung tissue, and urogenital system. Pathological processes can develop at sites of the agent location.

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