PRIMATOLOGY

Spontaneous Simian Mycoplasma Infection

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We present our many-year studies of spontaneous Mycoplasma infection in monkeys. Mycoplasma flora of healthy, acclimatized, and sick monkeys of different species is characterized. S ome characteristics (including pathogenic properties) of new Acholeplasma isolated from monkeys are described.

Key Words: *Mycoplasma infection; monkeys*

Studies of Mycoplasma infection in monkeys are very important for many reasons. First, 150 bacterial and parasitic agents common for monkeys and humans include *Mollicutes*, and therefore monkeys can transmit these agents, including pathogenic, to humans during contacts of any kind. Second, Mycoplasma often contaminate cell cultures derived from monkeys, which hampers virological studies and vaccine production on these cultures. In addition, Mycoplasma infection in both monkeys and humans is characterized by long-term persistence of the agent [2,4], which suppresses host immunity, activates and complicates processes induced by other agents in mixed infections. This can lead to death of these laboratory animals and distort the results of experiments. On the other hand, similarity of monkey and human reactions to Mycoplasma makes monkeys indispensable in studies of human Mycoplasma infections.

We studied and characterized Mycoplasma flora of monkeys of different species in health and disease and during acclimatization, and studied the course of infection on monkeys infected with new Mycoplasma species isolated for the first time.

MATERIALS AND METHODS

Mycoplasma infection was studied in 1313 healthy, acclimatizing, and sick monkeys of different species (Table 1).

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Smears from the oral mucosa, blood, parenchymatous organs, lymph nodes, and bone marrow specimens served as the material for microbiological studies. Smears from the oral mucosa and 10% organ suspension in normal saline were inoculated in nutrient media (0.3% agar and Hottinger broth) with 10-25% yeast extract: 20% equine serum and 500-1000 U/ml penicillin. The cultures were incubated at 37°C for 7 days. Ureaplasma were cultured in modified Taylor-Robinson medium [3], *M. hominis* and *M. pneumoniae* in medium B [8].

Sterol dependence, growth at 22°C, digitonin sensitivity, biochemical and hemolytic activities of the isolated cultures were evaluated. The strains were identified serologically in the growth inhibition test (GIT) in semisolid agar and by the filter paper disk method in solid media and by the immunofluorescent test (IFT) with standard antisera to Mycoplasma and Acholeplasma (Microbiological Associated) and with hyperimmune sera from rabbits immunized as described previously [3].

For evaluation of the prevalence of Mycoplasma infection in monkeys, 800 blood sera from monkeys of different species were examined (Table 2). The level of specific antibodies to different Mycoplasma and Acholeplasma was evaluated by the passive hemagglutination (PHA) test in the macro- and micromodifications.

Pathogenic characteristics of cultures isolated from monkeys (Acholeplasma strains 682 and 449 similar but not identical to *A. laidlawii*, freshly isolated from a monkey with spontaneous nephrosonephritis and

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from a monkey with hemoblastosis, and reference *A. laidlawii* strain) were studied on 7-8-day chick embryos (infected intravenously or into the yolk sac), BALB/c mice (intranasal infection), guinea pigs (intraperitoneal infection), and 16 monkeys (3 female rhesus macaques aged 2-3 years, 3 male *M. speciosa* aged 3-7 years, and 10 *Papio hamadryas* — 8 males and 2 females aged 7 months to 2 years). For infection, 3-day broth cultures of Acholeplasma with initial titer of 10^8 CFU/ml were used in the following volumes: 0.1 ml for chick embryos, 0.5 ml for mice, and 1 ml for guinea pigs. Monkeys were challenged with 3-day broth culture of freshly isolated strain, precipitated by ultracentrifugation, and concentrated 100-fold. Ten monkeys were inoculated by a combined method (intraperitoneally and intramuscularly) with strain 449 and 6 monkeys with strain 682 (Table 3). Monkeys infected with a single dose were observed for 1.5-13 months, after which they were sacrificed. Eleven months after the first inoculation 2 monkeys were repeatedly infected by the combined method in a dose of 22 billion bacterial cells one week before sacrifice. Nine *Papio hamadryas* were infected repeatedly (3-6 times) with 2.5-7-month intervals between inoculations. The animals were sacrificed 2 weeks to 2.5 months after the last injection. The period of observation was 12-35 months.

Control monkeys were injected with the same volumes of sterile culture medium. Observation of experimental and control monkeys included clinical, microbiological (re-isolation of the agent from different specimens at different terms of the experiment), and serological (evaluation of immune response) studies.

The animals were kept in isolated cages and had no antibodies to Mycoplasma, Ureaplasma, and Acholeplasma before the experiment. The animals were treated with tetracycline (orally, 10 mg/kg, twice a day) during 5 days before infection. The animals were sacrificed under calipsol anesthesia (ketamine hydrochloride0.1 mg/kg, Gedeon Richter).

Organ and tissue fragments for morphological studies were fixed in 10% neutral formalin, paraffin sections were stained with hematoxylin and eosin, in some cases periodic acid-Schiff reaction was carried out.

RESULTS

The incidence of Mycoplasma detection in the oral mucosa of healthy acclimatized animals depended on their species, the mean value being 6.7% (Table 1). It was maximum in *Er. patas patas* and *M. rhesus* (22.5 and 18%, respectively), low in rare monkey species (squirrel monkeys, *Theropithecus gelada*, baboons), and zero in *M. speciosa*. No Mycoplasma were detected in the blood and organs of healthy monkeys.

The incidence of Mycoplasma detection in the oral mucosa was slightly higher in the majority of acclimatizing newcomers and notably higher (up to 30-80%) in rare monkey species, but no Mycoplasma were detected from the blood and viscera (Table 1).

In sick monkeys Mycoplasma were isolated from the oral mucosa, blood, and viscera. The rate of Mycoplasma isolation from the blood and organs of *Papio hamadryas*, *Er. patas patas*, and *M. nemestrina* was even higher than from the oral mucosa, while in *M. speciosa* Mycoplasma were present in the blood and viscera, but not in the oral mucosa. Mycoplasma were most often isolated from the blood and viscera in monkeys with hemoblastosis, nephritis, and amyloidosis (*Papio hamadryas*, *M. speciosa*, and *Er. patas patas*), pneumonia, colitis, and tuberculosis (*M. nemestrina*, *M. mulatta*, and *M. speciosa*). Mycoplasma were repeatedly isolated from the oral mucosa and blood of some monkeys with chronic diseases (hemoblastosis, amyloidosis, nephritis) observed for several months (up to a year), which indicated long persistence of these agents.

The isolated cultures (a total of 291) were divided into 2 groups representing 2 families: sterol-dependent (Mycoplasma) and non-sterol-dependent (Acholeplasma); the latter group predominated (32.2 and 67.8%, respectively). Serological identification

TABLE 1. Incidence of Mycoplasma (% of Positive Tests) in Monkeys

Monkeys	Mouth	Blood	Organs
Healthy (*n*=480)	6.7	0	0
P. hamadryas (*n*=182)	1.1	—	—
M. mulatta (*n*=106)	18.1	—	—
M. speciosa (*n*=86)	—	—	—
M. nemestrina (*n*=57)	5.2	—	—
C. aethiops (*n*=16)	12.5	—	—
Er. patas patas (*n*=9)	22.5	—	—
other species (*n*=24)	1	—	—
Acclimatizing (*n*=196)	17.2	0	0
M. nemestrina (*n*=77)	8.2	—	—
M. mulatta (*n*=39)	20.3	—	—
M. fascicularis (*n*=20)	10.0	—	—
other species (*n*=50)	30-80	—	—
Sick (*n*=637)	24	13.1	15.2
P. hamadryas (*n*=450)	34.5	35.8	47
M. speciosa (*n*=80)	—	3.7	9.3
M. mulatta (*n*=60)	21.5	8.2	10.2
C. aethiops (*n*=6)	50	33	66
Er. patas patas (*n*=7)	43	71	71
Presb. cristata (*n*=14)	11.1	50	93
other species (*n*=20)	10	10	15

of the isolated cultures in GIT showed circulation of different Mycoplasma (*M. pneumoniae*, *M. salivarium*, *M. faucium*, *M. fermentans*, and *U. urealyticum*) and Acholeplasma (*A. laidlawii* and new serovars of this species) in the monkey population. The results of strain identification in GIT were confirmed by IFT. Some Mycoplasma were for the first time isolated from monkeys (*M. pneumoniae* from pig-tailed monkeys and rhesus macaques with pneumonia) or from some monkey species (*U. urealyticum* from baboons, *A. laidlawii* from *Er. patas patas*, *Papio hamadryas*, *Theropithecus gelada*, and baboons).

Antibodies to one or several Mycoplasma species were detected in 43.6% sera (Table 2).

Antibodies to *M. fermentans* were detected most often (14.4% animals); 10.1% monkeys had antibodies to Acholeplasma strain 682 which was isolated from monkeys with nephrosonephritis; *M. pneumoniae* ranked third (9.5%) and was the most prevalent in rhesus macaques (Table 2). Hence, the rate of infection with different Mycoplasma species depends on the monkey species; antibodies to simian Acholeplasma were detected almost as often as to the known human Mycoplasma species (in 25.5 and 28.6% animals, respectively). The antibody titers were not high (1:25-1:115) in tests with human Mycoplasma and notably higher (1:320-1:640) in tests with simian Acholeplasma antigens, which indicated more intense infection with them in comparison with human Mycoplasma.

In some monkeys Mycoplasma infection was asymptomatic, with antibody production and some clinical and morphological changes. For the first time in primatological literature we described a Mycoplasma infection caused by *M. pneumoniae* [14] which coursed as a respiratory disease with fever, dyspnea, cyanosis, peripheral blood leukocytosis, and lung involvement (interstitial pneumonia) in 13 pig-tailed monkeys during acclimatization. Three of these monkeys had renal disease (nephrosonephritis). Mycoplasma infection was concomitant with bacterial and viral infection in the majority of cases. The agent was isolated (with different frequency) from the lungs and blood and from virtually all examined organs.

One more disease, a peculiar type of nephrosonephritis with severe involvement of the tubular epithelium [2], was associated with a new Acholeplasma serovar (strain 682 related to *A. laidlawii*, isolated from the kidneys of 2 monkeys, *Papio hamadryas* and *M. nemestrina*). 10.1% of all monkeys and 24.8% *M. nemestrina* had antibodies to this strain. The characteristics of this strain and of Acholeplasma strain 449 isolated from monkeys with hemoblastosis, particularly their pathogenicity, were studied in detail in special investigations and compared with *A. laidlawii* reference strain.

Infection of chick embryos into the yolk sac with Acholeplasma led to the death of 10-40% embryos on days 5-14, except *A. laidlawii* infection. The respective Acholeplasma strains were reisolated in titers $10-10^5$ CFU/ml. Intravenous infection of embryos with these strains caused 100% embryonal death and the reference *A. laidlawii* strain caused 60% death on days 1-8 postinfection. Acholeplasma were reisolated in titers $10-10^7$ CFU/ml.

Acholeplasma tropism to lung tissue presenting as chronic interstitial pneumonia in albino mice and as desquamative interstitial giant-cell pneumonia in guinea pigs was observed in experiments on small laboratory animals. The disease was characterized by hematogenic dissemination, long (up to 60 days) persistence, and re-isolation of the agents from organs. Immunomorphological reaction was observed in immunogenesis organs; accumulation of specific antibodies in the blood was seen. These changes were less pronounced in infection with *A. laidlawii.*

Clinical morphological and serological studies of monkeys infected with new Acholeplasma species

TABLE 2. Incidence of Antibodies to Mycoplasma in Monkeys (Number of Animals)

Mycoplasma species	*P. hamadryas* (*n*=340)	*M. mulatta* (*n*=272)	*M. speciosa* (*n*=50)	*M. nemestrina* (*n*=105)	*M. fascicularis* (*n*=33)	Total (*n*=800)
M. fermentas	47 (13.8)	34 (12.5)	4 (8)	26 (24.8)	4 (12.1)	115 (14.4)
M. pneumoniae	21 (6.2)	39 (14.3)	7 (14)	6 (5.7)	3 (9)	76 (9.5)
A. strain 682	33 (9.7)	18 (6.6)	2 (4)	26 (24.8)	2 (6.1)	80 (10.1)
M. hominis	13 (3.8)	5 (1.8)	8 (16)	1 (0.9)	2 (6.1)	29 (3.6)
A. strain 548	23 (6.8)	36 (13.2)	5 (10)	2 (1.8)	2 (6.1)	68 (8.5)
A. strain 449	11 (3.2)	36 (13.2)	2 (4)	3 (2.8)	3 (9.1)	55 (6.9)
M. arthritidis	6 (1.7)	—	1 (2)	1 (0.9)	1 (3)	9 (1.1)
Positive tests, total	136 (42.2)	135 (50)	21 (42)	43 (41.8)	14 (42.4)	349 (43.6)

Note. The percentage is shown in parentheses.

TABLE 3. Results of Infection of Monkeys with Acholeplasma

Species, monkey No.	Strain, number of injections	Dose, 10^9 bacterial cells	Period of observation, months	Pathomorphology			Re-isolation of agent
				splenic index*, %	enlarged lymph nodes	organ involvement	
M. mulatta	**Strain 449**						
13896	One	42	1.5	0.33	+	—	+
14041	Ibid	42	13	0.17	—	—	—
14044	-»-	42	12	0.18	—	—	—
M. speciosa	**Strain 682**						
14247	One	22	3.5	0.08	—	Nephrosonephritis	+
14251	Two	44	11	0.12		Nephrosonephritis, cystitis	+
	Strain 449						
14255	Two	44	11	0.11	—	—	—
P. hamadryas	**Strain 449**						
14036	One	38	2.5	0.3	+	—	+
14030	Three	105	13.5	0.2	+	Nephrosonephritis	+
13729	Ibid	105	23	0.41	+	—	+
14157	-»-	105	23	0.25	—	—	+
14180	-»-	105	23	0.3	—	—	+
14138	Four	135	12	0.25	+	Lung lymphoma	+
	Strain 682						
13965	Three	105	26	0.28	+	Tonsillitis, nephrosonephritis	+
13411	Four	135	12	0.26	+	Nephrosonephritis, cystitis	+
13067	Five	165	20	0.15	—	—	+
13080	Six	195	35	0.2	+	Tonsillitis	+

Note. *Ratio of spleen to body weight. Normally no more than 0.2%.

demonstrated the development of chronic infection during the period of up to 3.5 months postinoculation (Table 3). Its main clinical symptoms were splenomegalia, lymphadenopathy, long (up to 3.5 months) persistence of Acholeplasma in the blood, and appearance of hemagglutinating antibodies (1:40-1:80 in *M. speciosa*, 1:160-1:320 in *Papio* after a single infective dose and 1.1280:1:2560 after repeated inoculations); the clinical status in general was satisfactory, no essential shifts were detected in the peripheral blood. Morphological analysis showed hyperplasia of the light centers of splenic follicles, lymph nodes (Fig. 1, *a*), lymphoid formations in the intestine, throat, lungs, increased counts of pyroninophilic cells in them, macrophagal reaction in the lymph node sinuses, and widening of the paracortical zones in them. These symptoms disappeared during later periods after infection with a single dose, antibody titers decreased, and the agent could no longer be isolated. The majority of animals inoculated repeatedly developed splenomegalia and lymphadenopathy after each subsequent inoculation. These symptoms were not observed in *M. speciosa* infected with both strains; immunological reaction in their immunogenesis organs was more likely to be suppressed. Renal involvement (a special type of nephrosonephritis with the predominant involvement of tubular epithelium, rejection of the apical part of cells, containing Schiff-iodic acid-positive incorporations pathognomonic for Mycoplasma) was observed in 1 of 10 monkeys infected with strain 449 and in 4 of 6 monkeys infected with strain 682. Segmented leukocytes, lymphocytes, macrophages, and cell detritus were seen in the lumens of the involved tubules. Lymphocytic infiltration was seen around the tubules. Glomerulonephritis with minimum changes or membranous glomerulonephritis with lymphoid infiltration round the glomeruli were observed.

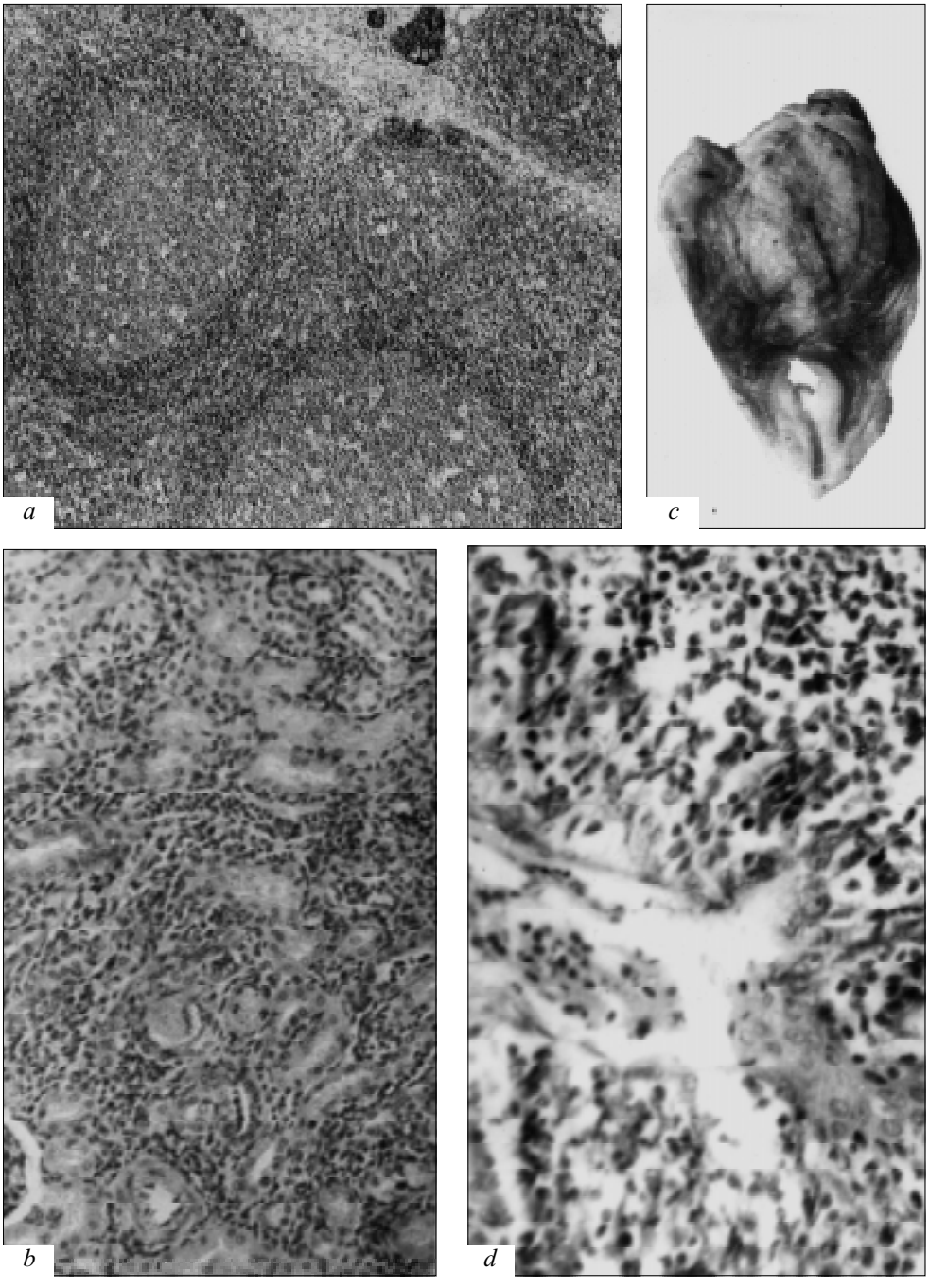

Fig. 1. Morphological changes in monkeys infected with simian Acholeplasma species. *a*) hyperplasia of lymph node follicles, extra follicle in perinodular fat (strain 449, monkey No. 13729), ×30; *b*) degeneration of tubular epithelium, lymphoid infiltration of intertubular tissue (strain 682, monkey No. 14251), ×80; *c*) cystitis (strain 682, monkey No. 13411); *d*) thinned epithelium of tonsillar crypt, protein detritus and desquamated cells in the lumen, edema (strain 682, monkey No. 13965), ×160. *a, b, d*: hematoxylin and eosin staining.

Hence, the strain isolated from monkeys with hemoblastosis was tropic for immune organs, lungs, and (rarely) for excretory organs. Acholeplasma strain isolated from animals with nephrosonephritis, in addition to tropism for immunogenesis organs, possessed clear-cut tropism for the urinary organs; experimental infection with this strain induced changes similar to spontaneous disease [2], which atteated to the etiological role of the isolated Acholeplasma in renal diseases of monkeys.

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