PRIMATOLOGY

Laboratory Diagnosis of Cytomegalovirus in Monkeys in the Adler Breeding Center and in Humans

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Monitoring of cytomegalovirus markers was carried out in the blood of humans and in the blood, lavage fluid specimens from the throat, and salivary gland tissues of monkeys of different species. Correlation between the percentage of cytomegalovirus infection and age was detected in humans. The virus was most often detected in salivary gland tissues and least so in the blood.

Key Words: *cytomegalovirus; monkeys; blood; salivary glands*

Cytomegalovirus (CMV) is a DNA virus from the *Herpesviridae* family, *Cytomegalovirus* genus [3,10]. Cytomegalovirus infection is highly prevalent and its manifestations are numerous, from asymptomatic course to grave generalized forms with involvement of the viscera and CNS [4,9]. A specific feature of CMV is a great variety of routes of infection: droplet spread, urogenital, intrauterine, postnatal, and through blood components in transfusion and organ transplantation. Cytomegalovirus infects organs and tissues, including nervous tissue, bone marrow, lymph node, liver, lung, gastrointestinal, genital, and blood cells. Primary infection can transform into the latent stage with subsequent probable reactivation during immunosuppression-associated conditions [6,8,11].

Along with human CMV, the viruses pathogenic only for their species (simian, guinea pig, rat, murine, *etc.*) are known, [1,2,5,10]. Comparative study of simian and human CMV can help us to understand specific features of infection and find approaches to prevention and development of specific therapy. By the present time, laboratory diagnosis of CMV of individual primate species is far from perfect.

The aim of our study was laboratory diagnosis of CMV in humans and monkeys, evaluation of the incidence of the virus in different biological materials, and evaluation of the efficiency of EIA and PCR.

MATERIALS AND METHODS

The study was carried out at Adler Breeding Center, Institute of Primatology. The objects of the study were 176 blood samples, 68 lavage fluid specimens from the throat, and 86 specimens of salivary gland tissues from monkeys of different species and blood specimens from 120 healthy humans. The blood, washout specimens from the throat, and salivary gland tissues were collected from different animals. The biological material from monkeys of different species was divided into 3 groups. Group 1 included blood specimens from 50 *Macaca mulatta*, 38 *Macaca fascicularis*, 30 *Papio hamadryas*, 20 *Papio anubis*, 30 *Cercopitecus aethiops*, and 8 *Macaca nemestrina*. Group 2 included throat lavage fluid specimens from 20 *Macaca mulatta*, 12 *Macaca fascicularis*, 13 *Papio hamadryas*, 6 *Papio anubis*, 14 *Cercopitecus aethiops*, and 3 *Ma-*

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TABLE 1. Antibodies to CMV in the Sera of Clinically Healthy Humans and Monkeys of Different Species

Group	Number of tested subjects	Seropositive	
		abs.	%
Normal subjects			
under 25 years	13	8	61
25-40 years	40	35	87
40-55 years	38	36	95
over 55 years	46	46	100
total	137	124	91
Healthy monkeys			
Macaca mulatta	50	15	30
Macaca fascicularis	38	12	32
Papio hamadryas	30	10	33
Papio anubis	20	5	25
Cercopitecus aethiops	30	11	37
Macaca nemestrina	8	2	25
total	176	55	31

caca nemestrina. Group 3 were salivary gland tissues from 25 *Macaca mulatta*, 15 *Macaca fascicularis*, 20 *Papio hamadryas*, 8 *Papio anubis*, 14 *Cercopitecus aethiops*, and *4 Macaca nemestrina*.

Extraction of DNA from the materials was carried out by two methods: guanidine thiocyanate (GuSCN) for extraction of DNA from the blood and from the throat lavage fluid specimens and by the proteinase method (with proteinase K) for DNA extraction from salivary gland tissues.

For extraction by the GuSCN method, the material (100 µl blood or lavage fluid specimen from the throat) was transferred into a tube with 300 µl 5 M GuSCN, 1% Triton X-100 (v/v), 20 mM EDTA, 50 mM Tris-HCl (pH 6.4), and 10 µl SiO_2, incubated for 30 min, centrifuged, and the precipitate was washed in 500 ml washing solution containing 5 M GuSCN and 50 mM Tris-HCl (pH 6.4) and twice washed in solution containing 10 mM Tris-HCl (pH 7.3), 50 mM NaCl, and 50% ethanol. DNA was eluted in 50 µl 0.1 M Tris-EDTA buffer (pH 8.3) [7].

For extraction by the proteinase method, the material (100-200 mg salivary gland tissue) was transferred into a tube with 1 ml solution containing 2.5 mM $MgCl_2$ and 20 µg proteinase K (30 U/mg), incubated (24 h at 54°C), proteinase was inactivated by heating (95°C, 5 min), the material was centrifuged (3 min at 13,000 rpm), and the supernatant was transferred into fresh tubes [7]. PCR amplification was carried out in 5 µl, the remainder was stored at -20°C.

Amplification was carried out in 25 µl solution of the following composition: 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 3.0 mM $MgCl_2$, 0.01% gelatin, 100 ng each primer, 0.2 mM each dNTP, and 2.5 U Taq DNA polymerase. Primers synthesized by Syntol Company were used. For positive control, DNA isolated from culture fluid of human fibroblast culture infected by human CMV AD169 laboratory strain was used. Distilled water served as the negative control. The PCR was carried out on a Terzik device (DNA Technologies).

After amplification, the material was applied to 2% agarose gel with 0.5 µg/ml ethidium bromide. Electrophoresis was carried out for 15 min at 50 A and 10 V/cm. Amplification products were visualized on a transilluminator at λ=330 nm.

TABLE 2. Results of PCR Testing of Blood Specimens from Clinically Healthy Humans and Monkeys of Different Species for CMV

Group	Number of tested subjects	CMV-positive	
		abs.	%
Humans	137	6	4
Macaca mulatta	50	2	4
Papio hamadryas	30	1	3
Macaca fascicularis	38	2	5
Cercopitecus aethiops	30	1	3
Papio anubis	20	1	5
Macaca nemestrina	8	–	0

TABLE 3. Results of PCR Testing Throat Lavage Fluid Specimens from Clinically Healthy Monkeys of Different Species for CMV

Species	Number of animals tested	CMV-positive	
		abs.	%
Macaca mulatta	20	3	15
Papio hamadryas	13	2	15.3
Macaca fascicularis	12	2	16
Cercopitecus aethiops	14	3	21
Papio anubis	6	1	17
Macaca nemestrina	3	–	0
Total	68	11	16

TABLE 4. Results of PCR Testing of Autopsied Salivary Gland Specimens from Monkeys of Different Species

Species	Number of tested animals	CMV-positive		CMV-negative
		abs.	%	
Macaca mulatta	25	16	64	9
Papio hamadryas	20	13	65	7
Macaca fascicularis	15	9	60	6
Cercopitecus aethiops	14	10	71	4
Papio anubis	8	4	50	4
Macaca nemestrina	4	2	50	2
Total	86	54	63	32

The serum from the whole blood of monkeys of different species and humans was used in EIA, which was carried out with commercial test system for detection of human IgG to CMV (Vector Best).

RESULTS

The reactivity of IgG to CMV was evaluated by EIA. The results of testing of 137 normal subjects are presented in Table 1. The incidence of CMV-seropositive specimens was 91%. A correlation between the age and presence of CMV antibodies was detected. Clinically healthy monkeys of different species were tested for antibodies to CMV by EIA (Table 1). The incidence of IgG to CMV in monkeys varied from 25 to 37% (3-fold less than in humans; Table 1).

Qualitative detection of the virus in different biological materials was carried out by PCR. Primers to the common conservative site of human and simian CMV gene UL56 were selected. The results of PCR testing of blood specimens from clinically healthy humans and monkeys indicate very low (0-5%) viremia level in both (Table 2).

Testing of throat washout fluid specimens showed another picture of CMV incidence (Table 3).

The total percentage of CMV-positive specimens was 16%, *i.e.* 4-fold higher than the results of blood specimens testing (Table 3). This can be due to possible latent infection of epithelial cells by the virus.

The final step of our study was CMV detection in simian salivary gland tissues (Table 4). The material was collected at autopsies.

The percentage of CMV detection in human and simian blood specimens by PCR was low, despite the presence of antibodies to the virus in 25-91% tested specimens. This fact indicates that virus charge in CMV carriership is too low to be detected by single-round PCR.

The incidence of virus detection in the throat lavage fluid specimens was 2-3-fold higher than in the blood, reaching 15-21%. This indicates latent infection of the epithelial cells in the throat by the virus.

The highest percentage of virus detection was found for the salivary gland tissues; the infection rate there reached 50-70%. This fact is explained by CMV tropism to salivary gland tissues and its latent persistence in these cells.

Relatively low percentage of CMV antibodies-positive monkeys in comparison with humans is presumably due to inter-species specificity of IgG and the difference in the antigenic structure of human and simian CMV. Therefore, human EIA test system cannot be used for the diagnosis of antiCMV antibodies in monkeys.

Hence, monitoring of CMV markers showed high percentage of infection in the presence of low virus charge, which is characteristic of latent infection.

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