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A seroprevalence study of primate workers for asymptomatic rhesus cytomegalovirus infection

J. Jason Bowman^a, Peter D. Burbelo^b, Rachel B. Gill^a, Michael A. Sauri^c, James M. Schmitt^d, and Jeffrey I. Cohen^{a,*}

^aMedical Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland ^bNeurobiology and Pain Therapeutics Section, Laboratory of Sensory Biology, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland ^cOccupational Health Consultants, Rockville, Maryland ^dOccupational Medical Service, Division of Occupational Safety and Health, Office of the Director, National Institutes of Health, Bethesda, Maryland

Abstract

Background—Human cytomegalovirus (HCMV) infection can cause severe disease in neonates and immunocompromised persons, and infectious mononucleosis in healthy adults. While, rhesus CMV (RhCMV) infects human cells in culture, it is unknown whether the virus can infect humans.

Objectives—We sought to determine whether primate workers, including those with injuries from animals, might be infected asymptotically with RhCMV.

Study design—We developed serologic assays that distinguish RhCMV from HCMV antibodies. We tested two groups of primate workers: those with documented injuries or mucosal splashes associated with rhesus macaques, and those with no documented exposure who worked with these animals.

Results—None of over 200 primate workers, including 119 with injuries or mucosal splashes associated with exposures to macaques, were seropositive for RhCMV.

Conclusions—The frequency of asymptomatic RhCMV infection in persons who work with rhesus macaques was <0.5% (<1/200 primate workers).

Keywords

Rhesus cytomegalovirus; cytomegalovirus; primate workers; zoonosis

*Corresponding author at: Laboratory of Infectious Diseases, Bldg 50, Room 6134, National Institutes of Health, 50 South Drive, MSC 8007, Bethesda, MD 20892-8007, Tel: 301-496-5265; fax: 301-496-8312. jcohen@niaid.nih.gov (J.I. Cohen).

Competing interests

All authors report no conflicts of interest relevant to this article.

Ethical approval

Ethical approval was given by the Office of Human Subjects Research at the National Institutes of Health.

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1. Background

Human cytomegalovirus (HCMV) is the major infectious cause of birth defects and the most important infection in transplant recipients. HCMV is the second most common cause of infectious mononucleosis.

Rhesus CMV (RhCMV), like its human counterpart, usually causes asymptomatic infections, but can result in congenital disease in neonatal animals and disseminated disease immunocompromised animals.¹ The genome of RhCMV is closely related to HCMV and at least 90% of RhCMV proteins have orthologs at the level of protein families.² RhCMV infects human fibroblasts, endothelial and epithelial cells in vitro.³ Thus, it is possible that the virus could infect humans.

Primate workers are potentially exposed to a number of rhesus macaque viruses and can be infected with rhesus B virus.⁴ Human infection with HIV, simian type D retrovirus, human T cell leukemia viruses 1–4, and simian foamy virus are thought to have originated from exposure to monkey tissues or blood.^{5–7}

2. Objectives

We sought to determine whether primate workers, including those with injuries from animals, might be infected asymptotically with RhCMV.

3. Study Design

Participants

Serum was obtained from the occupational health service provider for two cohorts of workers who handled primates as part of their routine evaluation. Serologic testing was performed in a blinded fashion. All sera were anonymized and the study was deemed exempt by the Office of Human Subjects Research at the National Institutes of Health. The first cohort was individuals who worked with rhesus macaques (primate workers), but for whom it was not known if they had incurred injuries involving rhesus macaques. The second cohort was primate workers with known injuries involving potential exposure to body fluids from rhesus macaques. Injuries included bites, mucosal splashes, lacerations, needlesticks, puncture wounds, and scratches. Human sera previously characterized as HCMV seropositive and seronegative⁸ were used for validating the specificity of the RhCMV assay.

Serologic assays

RhCMV proteins were cloned into plasmid pREN2⁸ to generate fusion proteins containing carboxyl terminal RhCMV proteins fused to Renilla luciferase gene. A portion of the extracellular domains of RhCMV glycoprotein H (gH) and glycoprotein O (gO) were amplified by PCR using primers RhCMV gH forward –5' **CCGGATCCCTGGAACCTACCGGCAG** and RhCMV gH reverse –5' **CCCTCGAGTCAACATGGACTGTAAAG**, RhCMV gO forward –5' **CCGGATCCAAGATCGCGGTAGCGCG**, and RhCMV gO reverse –5' **CCTCTAGAAATGGAATTCGGGGGAAA**. PCR products were digested with BamH I

(underlined) and Xho I (bolded) for RhCMV gH, or BamH I and Xba I (underlined and bolded) for RhCMV gO.

Antibody to RhCMV glycoproteins were measured using the luciferase immunoprecipitation system (LIPS) assay.⁸ Cos cells were transfected with RhCMV-Renilla luciferase constructs and lysates were prepared. To measure RhCMV antibody levels in animals, rhesus monkey plasma were diluted 1:10, and 1 ul was added to 1×10^7 light units (LU) of transfected Cos cell extract. Immunoprecipitations were performed by addition of protein A/G beads, and LU were determined by luminometry. A cut-off threshold limit was derived from the mean value plus 2 standard deviations of the background LU. All LU data shown represent the average of at least two independent experiments. Tests for RhCMV were considered positive if antibody to either RhCMV gH or gO were positive for a given sample. A standard assay for RhCMV, which is expected to cross-react with HCMV, was used to identify RhCMV-positive monkey serum (Virus Reference Laboratory, San Antonio, Texas), while a LIPS assay validated for HCMV, which likely cross-reacts with RhCMV was used to identify HCMV-positive human serum.⁸

4. Results

A RhCMV serologic assay was developed that was specific for RhCMV and did not cross-react with HCMV. RhCMV glycoproteins expressed on the surface of the virus were compared with their HCMV homologs, and portions of RhCMV glycoproteins were chosen that share little homology (gH, gO) with HCMV. Portions of these RhCMV glycoproteins were cloned as fusion proteins with Renilla luciferase, sera from primate workers were used in immunoprecipitations, and luciferase activity was measured. Sera from RhCMV-positive rhesus macaques were used as positive controls for RhCMV antibody to test the sensitivity of the assay, while sera from a RhCMV-negative macaque and from HCMV-positive and HCMV-negative persons who had no known exposure to rhesus macaques were used as negative controls for RhCMV antibody to test the specificity of the assay. Using a combination of constructs expressing RhCMV gH and gO in our LIPS assay, we detected RhCMV antibody in 64% (7/11) of RhCMV-positive monkeys (based on a conventional assay), but in none of the known HCMV-positive or HCMV-negative humans (Table 1).

We used the RhCMV-specific LIPS assay to test two cohorts of primate workers. None of the 91 primate workers for whom we were unable to obtain a history for potential exposure to primate body fluids were positive for RhCMV (Table 2). We then tested 119 primate workers with injuries or mucosal splashes associated with rhesus macaques. Two samples were tested from each worker; one was obtained at the time of the injury and the second was obtained a median of 49 days later (86% were 28 days after injury). None of 119 workers with documented bites, scratches, lacerations, puncture wounds, needlestick injuries, or mucosal splashes involving rhesus macaques were seropositive for RhCMV (Table 2). To verify that sera from primate workers had antibody detectable by the LIPS assay, we tested sera from all 119 individuals from the second cohort for antibody to HCMV. Fifty-one (43%) sera tested had antibody to HCMV. This is similar to the HCMV seroprevalence rate of 50% in the United States reported from 1988–2004.⁹

5. Discussion

We did not detect RhCMV infection in primate workers based on serologic assays. RhCMV is ubiquitous in rhesus macaques with seropositivity rates nearly 100% at one year of age.¹ RhCMV is shed from the saliva, urine, and genital secretions of healthy infected rhesus macaques and sustained viremia can occur with immunosuppression.¹¹ Therefore, we looked at sera from persons who worked with rhesus macaques who had no documented exposures to body fluids from the animals as well as from other persons that had documented injuries including bites, scratches, lacerations, puncture wounds, needlestick injuries, or splashes to the mucosa with fluids from rhesus monkeys. Similar injuries or mucosal splashes involving B virus, another herpesvirus endemic in rhesus macaques, have resulted in human infections which have often been fatal.⁴ B virus, like RhCMV, is present in saliva and genital secretions of most macaques. Despite hundreds of macaque bites and scratches that occur in primate workers each year in the United States, B virus infection of humans is very rare. Thus, if infection rates of B virus and RhCMV in humans are similar, it is possible that our sample size (210 persons) may not have been sufficient to detect RhCMV infection of humans. Alternatively it is possible that a more sensitive test for RhCMV, or testing more individuals with frequent exposure to rhesus saliva, urine, or genital secretions might have identified RhCMV-positive persons.

HCMV infection of healthy CMV-seronegative adults can result in infectious mononucleosis.¹² If RhCMV infected primate workers, similar symptoms might occur. If the HCMV ELISA assay is not specific for HCMV, these individuals might be diagnosed with HCMV mononucleosis when in fact their disease could be due to RhCMV.

While the frequency of RhCMV infection based on serostatus in persons who work with rhesus macaques (many of whom had injuries associated with the animals) was <0.5% (<1/200 primate workers) in our study, vigilance is still required to avoid exposures when working with macaques since B virus and other zoonotic infections can occur.

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Table 1

Rhesus and human cytomegalovirus serologic results using rhesus cytomegalovirus-specific LIPS assay

Serum	Number Positive	Number Tested (% positive)
RhCMV known seropositive ^a	7	11 (64)
RhCMV known seronegative ^b	0	1 (0)
HCMV known seropositive ^b	0	20 (0)
HCMV known seronegative ^b	0	11 (0)

^aPositive control for rhesus cytomegalovirus antibody assay^bNegative control for rhesus cytomegalovirus antibody assay

Table 2

Rhesus cytomegalovirus serologic results in primate workers

	Number Positive	Number Tested
Type of Exposure		
Unknown	0	91 samples
Bite	0	10 paired samples ^a
Mucosal splash	0	22 paired samples ^a
Laceration	0	23 paired samples ^a
Needlestick	0	11 paired samples ^a
Puncture wound	0	5 paired samples ^a
Scratches	0	48 paired samples ^a
Total	0	210

^a Paired samples indicate two samples from the same subject; one was obtained at the time of the injury and one was obtained a median of 49 days later.