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Cytomegaloviral hypophysitis in a simian immunodeficiency virus infected rhesus macaque (*Macacca mulatta*)

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Abstract

Rhesus macaques experimentally infected with Simian Immunodeficiency Virus (SIV) experience immunosuppression and often opportunistic infection. Among the most common opportunistic infections is rhesus cytomegalovirus (RhCMV), a ubiquitous betaherpesvirus that undergoes continuous low-level replication in immunocompetent monkeys. Upon SIV-mediated immunodeficiency, RhCMV reactivates and results in lesions in numerous organ systems including the nervous and reproductive systems. We report the first case of cytomegaloviral hypophysitis in a SIV-immunocompromised rhesus macaque.

Keywords

non-human primate; CMV; cytomegalovirus; pituitary; model; viral; inclusions; intra-nuclear; intra-cytoplasmic

Introduction

Cytomegaloviruses (CMV) are betaherpesviruses that infect humans and a wide range of non-human primate species including *Macaca mulatta*, *Saguinus fuscicollis*, *Saimiri sciureus*, *Mandrillus leophaeus*, and *Papio spp.*¹⁻⁴ Animals shed CMV through saliva, urine, semen, cervical secretions, and breast milk; most group-housed animals are exposed to CMV within the first year of life.⁵⁻⁷ Rhesus cytomegalovirus (RhCMV) has a reported seroprevalence of 100% in animals older than one year at the California National Primate Research Center, and 95% within wild Indian populations.^{5,8} Despite the high seroprevalence of CMV in rhesus macaques, clinical manifestation is infrequent in immunocompetent animals. Unlike other herpesviruses that undergo periods of latency,

CMV replicates continuously in infected tissues.⁹ The balance between viral replication and immune response results in low-level viremia and a lack of clinical signs.⁸

Human cytomegalovirus (hCMV) is epidemiologically similar to its simian counterpart. Approximately 10% of human infants are infected with hCMV by six months of age.¹⁰ Up to 40% of infants that nurse for longer than one month from seropositive mothers become infected.¹¹ Increased hCMV seropositivity is seen in populations where individuals live in close proximity.¹² The widespread distribution of viral infection in these communities mirrors that of rhesus macaque colonies, making macaques a good model for studying hCMV.

Similar to hCMV in immunosuppressed humans, RhCMV may result in clinical disease in rhesus macaques immunocompromised by simian immunodeficiency virus (SIV) infection. In SIV infected macaques, the severity of clinical disease correlates with SIV-induced immune suppression and an increase in plasma RhCMV DNA.¹³⁻¹⁶

RhCMV lesions in SIV infected animals are widespread and reported in the central and peripheral nervous systems, gastrointestinal tract, reproductive tract, and respiratory system. RhCMV has a predilection for neurologic and reproductive tissues.^{17,18} Within the peripheral nervous system, lesions have been demonstrated in the pulmonary, hepatic, mesenteric, penile, facial, sciatic, periocular, and trigeminal nerves.^{17, 19-21} In a review of SIV-RhCMV co-infected rhesus macaque lesions, the meninges were the most consistently and severely affected tissue.¹⁷ However, hypophyseal infection with RhCMV has not been previously reported. We report the first case of RhCMV infection of the pituitary gland in a SIV-infected rhesus macaque.

Case report

Clinical presentation

A 4.6kg, six-year-old SIV infected female rhesus macaque (*Macaca mulatta*) was admitted for weakness, ataxia, epistaxis, and intermittently lying down in her cage. The animal had received multiple intravaginal doses of SIVmac239 at 1000 TCID₅₀/1ml/week for 5 weeks, then 5000 TCID₅₀/1ml/week for 5 weeks, and then 10,000 TCID₅₀/1ml/week until the animal was confirmed SIV-infected (plasma viral load > 500 copies/ml for 2 consecutive weeks). The animal was confirmed infected after 6 inoculations at 10,000 TCID₅₀/ml. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the SNPRC. The SNPRC is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. There was no clinical history prior to study enrollment. She was specific pathogen free for simian T-lymphotrophic virus 1 (STLV1) and 2 (STLV2), simian retrovirus 2 (SRV), SIV, and macacine herpesvirus 1 before infection with SIV. A complete blood count (CBC), clinical chemistry, and cerebrospinal fluid (CSF) analysis were performed. The CBC revealed a mild hypochromic, normocytic anemia; lymphopenia; and monocytosis. The serum chemistry revealed hypoproteinemia, elevated BUN/creatinine ratio, and a metabolic alkalosis. The CSF analysis was within normal limits. Due to the patient's deteriorating clinical condition, established study end points, and poor prognosis, euthanasia was elected and the animal was submitted for

necropsy. At the time of euthanasia, the animal was SIV-infected for only 14 weeks (3 months) with a plasma viral load of 126,526,833 copies/ml and CD4 count of 1810 CD4/mm (Calculated).

Pathologic Findings

No gross lesions were identified. Representative samples were fixed in 10% neutral buffered formalin and five micron H&E sections were examined. Histologic lesions were restricted to the pituitary gland with no other tissues affected. Areas of necrosis and neutrophilic inflammation surrounded the neurohypophysis and extended into the adenohypophysis. Degenerate cells within this area had large eosinophilic intranuclear inclusions and/or small eosinophilic cytoplasmic inclusions (Fig 1).

Immunohistochemistry

Immunohistochemistry was performed using a polyclonal antiserum for the RhCMV immediate-early protein (IE1) (Gift from Peter A. Barry, UC Davis) as previously described with modifications using the IHC automated Leica BondRx platform according to the manufacturer's recommendations.²² CMV positive cells were found within the pituitary gland and adjacent neuropil (Fig 2).

Discussion

Hypophysitis in this report is consistent with RhCMV reactivation following SIV-induced immunosuppression and represents the first report of such lesion in rhesus macaques.^{16,17,19-21,23-28} Similar lesions have been seen in AIDS patients with hCMV, suggesting an additional parallel to human disease.²⁹⁻³¹ Human cases of cytomegaloviral hypophysitis are most commonly identified as incidental findings in 4-5% of autopsies.³² Ferreiro reported three cases of hCMV infection in the anterior lobe of the pituitary in AIDS patients; none of which had any significant inflammation or necrosis of the gland.³⁰ Pituitary necrosis was observed in 10 of 88 patients, however none of these were associated with CMV.³⁰ Sano et al. reported five cases of hypophyseal hCMV infections in AIDS patients; only one had a necrotic area that was associated with inflammatory infiltrate.²⁹ All cases in both of these reports^{29, 30} had evidence of systemic CMV infection; this differs from the findings in this macaque where lesions only involved the pituitary gland. The presence of CMV associated hypophyseal inflammation and necrosis observed in this animal also appears to be uncommon in the human cases.^{29, 30} Clinical signs of hypophysitis in humans most commonly include headaches and visual disturbances,³³ which are thought to relate to localized distention and inflammation. Other clinical signs are related to deficits in hormone production, mainly including adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), gonadotropins, and prolactin (PRL).³³ The clinical signs in this case were non-specific and not definitively associated with the hypophysitis. The changes seen on bloodwork could be related to hormonal dysregulation secondary to hypophysitis, but without additional hormonal assays we cannot rule out SIV-associated comorbidities as the cause of the clinical presentation. Ultimately, further study of rhesus macaques and SIV-RhCMV coinfection is necessary to better understand the pathogenesis of CMV-associated neurologic disease in immunosuppressed individuals.^{6,8,14,15,23}

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Figure 1.

The pituitary is largely replaced by necrosis and neutrophilic inflammation. Note eosinophilic intranuclear inclusions (N) in the adenohypophysis and the areas of necrosis. Note the small eosinophilic cytoplasmic inclusions (C) in the areas of necrosis. Bar = 50 μ m.

Figure 2. Pituitary gland, rhesus macaque. RhCMV-induced hypophysitis characterized by the presence of numerous immunohistochemically positive cells (arrows) for CMV-IE1 (DAB; brown). Bar = 50 μ m.