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Dyscoria Associated with Herpesvirus Infection in Owl Monkeys (*Aotus nancymae*)

Alfonso S Gozalo,[1,*,†] Enrique J Montoya,[1] and Richard E Weller[2]

Dyscoria was noted in a female owl monkey and 2 of her offspring. The third offspring was found dead with necrohemorrhagic encephalitis. Two male monkeys paired with the female died, 1 of which showed oral ulcers at necropsy. Histologic examination of the oral ulcers revealed syncytia and eosinophilic intranuclear inclusion bodies in epithelial cells. Ocular examination revealed posterior synechia associated with the dyscoria in all 3 animals. Serum samples from the female and her offspring were positive for *Herpesvirus simplex* antibodies by ELISA. The clinical history, gross and microscopic lesions, and serology results suggests a herpesviral etiology, possibly *H. simplex* or *H. saimiri 1*. This report underscores the risks associated with introducing into breeding or research colonies animals that previously were kept as pets or those from unknown origin that could carry asymptomatic pathogenic *Herpesvirus* infections. In addition, herpesviral infection should be considered among the differential diagnoses if dyscoria is noted in nonhuman primates.

Abbreviations: EBV, Epstein Barr virus; HHV6, human herpesvirus 6; HSV1, herpes simplex virus type 1; HZO, herpes zoster ophthalmicus

Most primates carry their own *Herpesvirus* species, which normally do not cause clinical disease in their natural host. However, when these viruses infect a different primate species, they can cause significant clinical disease or even death.[1] Clinical signs and lesions vary depending on the species of *Herpesvirus* and primate involved. In the case of α herpesviruses, clinical signs commonly include localized vesicles and ulcers affecting the skin, oral mucosa, conjunctiva, and external genitalia; encephalitis and disseminated fatal disease also have been observed in some cases.[1] Ocular lesions, mainly keratitis,[1,13] have been described, and anisocoria has been reported recently.[8] However, to our knowledge, dyscoria ascribed to herpesvirus infection in humans or nonhuman primates has not previously been reported. Here we describe dyscoria associated with a herpesviral infection in an owl monkey colony.

Case Study

An adult female owl monkey (*Aotus nancymae*) was received at our institution (the Center for Reproduction and Conservation of Nonhuman Primates, Iquitos, Peru) as a donation. No clinical history was provided with the animal except that the monkey was a former pet. Housing conditions and husbandry at the Center have been described elsewhere.[5] Briefly, the owl monkeys were housed as breeding pairs with their offspring in $2 \times 1 \times 1$ m cages, were fed biscuit prepared inhouse, and were kept under natural temperature and photoperiod conditions (the Center is located in the tropical rainforest of Peru). All animal procedures were approved by the Universidad Nacional Mayor de San Marcos institutional guidelines on animal care and use. Quarantine procedures for newly arrived monkeys included a 30-d quarantine, during which the animals were physically examined, weighed, tattooed, tuberculin-tested, and dewormed with thiabendazole and underwent parasitologic evaluation by means of direct fecal exams and fecal flotation methods. If diarrhea was noted, fecal samples were collected and cultured for pathogenic enterobacteria, and the animal was treated accordingly.

The index monkey was clinically healthy on arrival and, after an uneventful quarantine, was introduced into the colony and paired with an adult male *A. nancymae* (male 1). Six months later, the female monkey gave birth to a healthy newborn. Three months after that birth, the adult male monkey (male 1) was found dead; no gross lesions were present at necropsy. Because cardiac disease is common in owl monkeys, it was suspected as the cause of the sudden death.[4] After the newborn was weaned, the female monkey was paired with another adult male (male 2), but they were separated the next day because of fighting.

The female monkey then was paired with a third male (male 3) and gave birth again 7 months later. Male 3 was found dead shortly after the female gave birth. Weight loss and ulcerative stomatitis affecting the hard palate were the main gross findings at necropsy. Because owl monkeys are nocturnal and because several animals were in the same cage, decreased food consumption was not noted, and the animal may have died from inanition, as we suspect that the oral lesion was painful and prevented the animal from eating and possibly drinking. Tissue samples from the oral lesion and major organs were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin for light microscopy. In addition, samples from the hard palate were cultured in blood, nutrient, McConkey, and Saboureaud agars under aerophilic conditions, incubated at 37 °C, and read at 24, 48, 72, 96, and 120 h after plating. Colonies were identified by using routine differential biochemical media.

The female monkey was paired with a fourth male (male 4), giving birth 6 mo later to a third offspring, which was found

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[1]Instituto Veterinario de Investigaciones Tropicales y de Altura, Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos, Iquitos, Perú; [2]Pacific Northwest National Laboratory, Richland, WA
[†]Present address: Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, and SoBran Incorporated, Bethesda, MD
[*]Corresponding author. Email address: gozaloa@niaid.nih.gov

dead at 5 wk of age. At necropsy, necrohemorrhagic encephalitis affecting the frontal lobe was the main finding. However, severe autolysis precluded histologic examination. Histologic examination of the brain was not attempted because neural tissue is most sensitive to hypoxia manifesting marked cellular changes soon after death due to rapid autolysis.[6] The infant monkey most likely died late in the afternoon after working hours and was found the next morning. Because the primate colony is in the jungle and outdoors, the hot and humid weather leads to rapid decomposition of carcasses.

At approximately 1 y of age, both of the first 2 offspring were found to have bilateral irregularly shaped pupils (Figure 1). On closer examination, the dam herself was found to have a mildly irregular left pupil; the abnormality in shape became more noticeable when the monkey was exposed to bright light (Figure 1). The tentative diagnosis for the abnormal pupil shape was iris coloboma. The female and her living offspring were anesthetized with ketamine hydrochloride (10 mg/kg body weight) and examined by a board-certified ophthalmologist using a slit-lamp microscope. Examination revealed posterior synechia associated with the irregular pupil in all 3 animals, changing the diagnosis to dyscoria. Histologic examination of male 3, who died with ulcerative stomatitis, showed marked tissue necrosis with a mixed inflammatory cell infiltrate. Epithelial cell syncytia, with margination of nuclear chromatin and irregular eosinophilic inclusion bodies extending to the borders of the nuclear membrane, was observed at the periphery of the lesion (Figures 2 and 3). Other histologic findings were mild interstitial myocardial fibrosis and chronic nephropathy, both common conditions in owl monkeys.[4] Microbiologic cultures revealed *Candida* spp.; *Candida* was not considered to be the main pathogen because no yeast cells were found on histologic examination of the ulcerated lesion. These results suggested a viral etiology compatible with herpesviral infection.

Because the index case (the female monkey) was a former pet donated to the primate center, the first rule out to be considered in the differential diagnosis was *Herpesvirus simplex* infection. Serum samples from the female monkey, her offspring, and 2 unrelated clinically healthy *A. nancymae* (as negative controls) were submitted to a reference laboratory (Instituto Nacional de Salud, Lima, Peru) for ELISA testing for *H. simplex*. The serum samples from the female monkey and her offspring were positive for *H. simplex* antibodies, whereas the 2 control animals were *H. simplex* antibody-negative. Because *H. simplex* is highly infectious and can cause high morbidity and high mortality in owl monkeys, a total of 7 animals including the female monkey, her 2 offspring, and 4 monkeys that had been in physical contact with the infected animals were euthanized. All animals were clinically healthy and, apart from dyscoria, no other lesions were noted at necropsy. No similar cases have since occurred in our owl monkey colony.

Discussion

Herpesvirus simplex (HSV) is an α herpesvirus capable of causing latent or active infections in humans, which are the natural reservoir. Oral lesions and encephalitis occasionally occur with HSV1 infections, whereas genital lesions in adults and disseminated disease in children are more typical of HSV2 infection.[16] Human-to-monkey and monkey-to-monkey transmission have been described.[1] Clinically, lesions in nonhuman primates can be local or generalized; oral vesicles and ulcers, conjunctivitis, encephalitis, and death may occur.[1] Histologically, multinucleated cells, syncytia, and intranuclear inclusion bodies associated with the lesions can be found.[1,13] Owl monkeys, tree shrews, lemurs, marmosets, and tamarins are susceptible to generalized disease characterized by focal necrosis and hemorrhage in all organs, usually with intranuclear inclusion body formation.[1,8,13] Chimpanzees and gibbons can be infected with HSV, but the infection usually remains confined to skin, oral cavity, external genitalia, and conjunctiva.[1,12] Recently, however, encephalitis associated with HSV1 infection has been described in gibbons that had oral, lingual, labial, or genital vesicles and ulcers associated with conjunctivitis and keratitis.[7,14] Anisocoria was found recently in a 5-y-old male *Callithrix geoffroyi* that died during an HSV outbreak in a marmoset colony at a French zoo.[8] However, to our knowledge, dyscoria has not previously been ascribed to herpesviral infections in nonhuman primates. During systemic infections, chemical mediators of inflammation may affect ocular endothelial permeability and function, allowing inflammatory cells to migrate from the iris vasculature into the aqueous humor. Proteins, particularly fibrin, can leak from the ciliary body and iris vasculature and predispose to the formation of posterior or peripheral anterior synechia.[6] In our cases, the monkeys might have had a herpesviral nonsuppurative uveitis that resolved but resulted in formation of posterior synechia and the observed pupillary irregularity.

Because the clinical signs and lesions of owl monkeys with HSV are indistinguishable from those caused by *Herpesvirus saimiri 1* (herpes T) infection and because ELISAs do not discriminate between α herpesviruses, the cases we describe in this report might have been due to infection with *H. saimiri 1*.[2,9,15] However, the history of the index case (the female monkey) suggests close contact with humans and possible exposure to HSV. Immunohistochemistry or PCR tests on DNA extracted from formalin-fixed or paraffin-embedded tissues using primers specific to the polymerase gene region can distinguish among various α herpesviruses that are indistinguishable by serology.[7] The present cases occurred over a span of almost 2 y since the first male owl monkey died, because the mortality was low, there was no clear indication of what might have been the cause of death or whether there were a connection between the deaths until we received the histology report for male 3 and until animals in the colony were tested for herpesviruses. As soon as the infection was confirmed, the decision was made to euthanize the affected animals immediately and all those monkeys that had physical contact with the herpesvirus-positive cases to prevent further spread of the infection in the colony. Archived formalin-fixed tissues or paraffin-embedded blocks were not available from the owl monkey with oral ulcers.

As mentioned earlier, because ELISA does not distinguish between α herpesviruses, the infection in these animals could be due to any α herpesvirus. However, because of a history of close contact with humans, the characteristics of the lesions observed (oral ulcers and encephalitis) and serology results, the most likely agent was HSV. However, *H. saimiri 1* must also be included in the differential diagnosis because the clinical signs and pathologic lesions of both agents are indistinguishable in owl monkeys and because no detailed records were available for the index case (the female monkey), which might have been in close contact with other monkey species while held in captivity.

Other α herpesviruses, such as herpes zoster virus, can cause ocular lesions in humans.[10] However, herpes zoster virus is less likely to be the cause of the dyscoria in the owl monkeys in the present report in light of several considerations. First, herpes zoster infection presents with pruritus and a generalized pustular–vesicular rash that involves the deep dermis of the skin in contrast to herpes simplex, which is limited to the epidermis.[10] No skin pustular–vesicular rash was noted

Figure 1. (A) *A. nancymae.* First offspring showing bilateral dyscoria. The posterior synechia impedes normal response of the iris to bright light, resulting in irregular pupil shape. (B) *A. nancymae.* Second offspring showing marked bilateral dyscoria at approximately 1 y of age. (C) *A. nancymae.* Index case. Adult female monkey showing mild dyscoria in the left eye, which was much more noticeable when exposed to bright light. Note the healed nose lesion due to fighting with male 2.

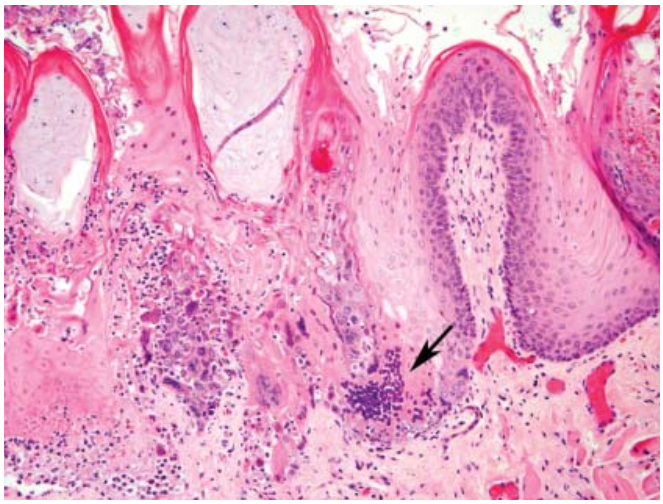

Figure 2. *A. nancymae.* Adult male 3. Hard palate ulcer. Note cell necrosis and epithelial cell syncytia at the periphery of the lesion (arrow). Hematoxylin and eosin; magnification, ×100.

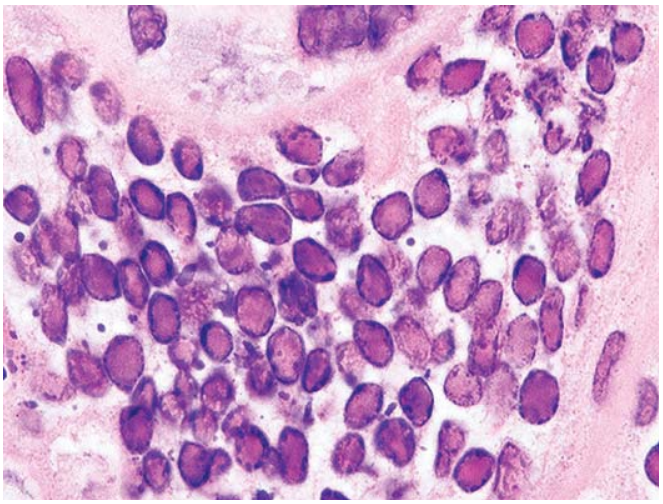

Figure 3. *A. nancymae.* Adult male 3. Hard palate ulcer. High-magnification image showing epithelial cell syncytia. Note the margination of nuclear chromatin and irregular eosinophilic inclusion bodies extending to the borders of the nuclear membrane. Hematoxylin and eosin; magnification, ×1000.

before or after death in the owl monkeys in the present report. In addition, in the owl monkey that showed oral ulcers, these lesions were confined to the epithelial lining. Second, in humans, herpes zoster ophthalmicus (HZO) manifests in the early stages of the disease with episcleritis or scleritis that may become persistent. All of the corneal inflammatory conditions in HZO can give rise to lipid keratopathy and facet formation. Interstitial keratitis can result from any long-term herpes zoster corneal inflammation condition and usually results in extensive corneal vascularization that subsequently leads to lipid deposition, scarring, and possible perforation.[10] In contrast to what is reported in HZO, no corneal lesions were found in the owl monkeys in the present report. Third, in HZO, unilateral anterior uveitis in association with a specific vascular occlusive sectoral iris atrophy and no evidence of prior or concurrent epithelial or stromal keratitis is a common presentation.[10] Although this lesion might clinically resemble the dyscoria observed in the owl monkeys in the present report, their dyscoria was not due to iris atrophy (as in HZO) but to posterior synechia. Fourth, other lesions reported in HZO that were not observed in the owl monkeys in this report are retinal perivasculitis, ischemic optic neuritis, and necrotizing retinopathy.[10] Finally, herpes virus zoster has previously been reported to occur in apes but not in monkeys.[11]

The likelihood of another herpesviruses, including Epstein–Barr virus (EBV), *H. saimiri 2*, *H. ateles*, and human herpesvirus 6 (HHV6), as the cause of the clinical signs observed in the owl monkeys in this report is small. EBV, *H. saimiri 2*, and *H. ateles* have been well studied in nonhuman primates and are known to cause malignant lymphoma and leukemia in owl monkeys and other New World monkeys.[11] Further, HHV6 is an oncogenic herpesvirus that causes lymphoproliferative disorders in humans.[3] In the owl monkeys in the present report, no lymphoma or other lymphoproliferative disorders suggestive of EBV, *H. saimiri 2*, *H. ateles*, or HHV6 infection were observed. In addition, EBV, *H. saimiri 2*, and *H. ateles* are γ herpesviruses and HHV6 is a β herpesvirus and therefore were unlikely to cross-react with α herpesviruses antibodies in ELISAs.

Other nonherpesviruses that cause syncytia, such as measles, were considered unlikely because measles is a member of the Paramyxoviridae. This family of viruses is known to cause giant cell pneumonia in owl monkeys,[1] but no pneumonia was diagnosed in any of the owl monkeys in the present report. In addition, measles does not cross-react with α herpesviruses antibodies in ELISAs.

No retrospective seroconversion studies were possible in the owl monkeys in this report because sequential archived

serum samples were unavailable. At the time of the outbreak, the colony contained approximately 1700 monkeys, and routine sequential serum banking for all animals in the colony was not performed due to logistical limitations. However, because no clinical cases compatible with herpesviral infection had occurred in the colony before the arrival of the female monkey, we believe that she arrived already infected and that the offspring were infected either in utero or shortly after birth.

In the present report, the clinical history, gross and microscopic lesions, and serology results are compatible with α herpesviral infection, possibly *H. simplex* or *H. saimiri 1*. Other α herpesviruses, although possible, were less likely to be the cause of the clinical signs in the presented owl monkeys, as discussed earlier. A search of the scientific literature failed to reveal any previous report of dyscoria associated with *Herpesvirus* infection in nonhuman primates. This report underscores the risks associated with introducing animals into breeding or research colonies that were previously kept as pets or those from unknown origin that could carry asymptomatic pathogenic herpesviral infections. In addition, herpesviral infection should be considered among the differential diagnoses if dyscoria is observed in nonhuman primates.

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