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Oral Papillomas and Papilliform Lesions in Rhesus Macaques *(Macaca mulatta)*

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Oral papillomas in two male rhesus macaques that were diagnosed morphologically as filiform and squamous types are described. Two additional macaques had oral papilliform lesions consistent histologically with papillary hyperplasia. Immunohistochemistry, along with electron microscopy and PCR assays, failed to demonstrate evidence of papillomavirus in any of the tumors; however, such results are often lacking when suspect oral lesions in humans and other species are assessed. Other potential causes of the papillary masses include chronic irritation and perhaps a genetic susceptibility. Benign tumors of the oral epithelium in macaques have not been reported previously; they appear to be rare and of variable clinical significance.

Benign oral tumors of the stratified squamous epithelium in human patients may be associated with human papillomavirus, trauma, genetic background, or other factors (2, 4, 7, 12). Those of viral origin are of particular concern because of the potential for malignant transformation. Histological and clinical history criteria typically are used to categorize the various types of oral epithelial lesions in humans, although molecular biology techniques and immunohistochemistry are also available for research purposes. In contrast to those in humans, such oral growths have rarely been reported in nonhuman primate species, and the only published confirmation of papillomaviral etiology has been in chimpanzees with focal epithelial hyperplasia (3, 10, 13). In part because vesicular lesions resulting from Cercopithecine herpesvirus 1 infection may be manifest within oral mucosa, the oral cavities of captive rhesus macaques (*Macaca mulatta*) periodically are evaluated by laboratory animal veterinarians. But whereas squamous cell carcinoma has been described previously in this species, oral tumors arising from the epithelium are recognized infrequently. In the present report, four cases in rhesus monkeys that exhibited characteristics of oral papillomas and papillary hyperplasia are discussed.

Case Reports

Rhesus 1. The adult male rhesus macaque participated in cognitive neuroscience studies at the Massachusetts Institute of Technology (MIT) and was singly housed there. Three years after its arrival at the animal facility, physical findings were unremarkable except that an oral examination revealed a pale, frondlike mass located on the midline at the juncture of the hard and soft palates. Individual fingerlike projections averaged 5 mm in length and were joined at the base, which was approximately 4 mm in diameter. The mass was surgically excised. No recurrence has been apparent over 7 years of followup.

Rhesus 2. Oral lesions were assessed in this male macaque 6 years after its birth at the New England Primate Research Center (NEPRC). The animal lived in gang housing and was relocated to a new group several times. Blood and bone marrow aspirate samples were collected occasionally, but rhesus 2 did not undergo any experimental procedures. When first examined and biopsied, proliferative gingival tissue extended over labial and lingual alveolar areas of both the upper and lower jaws, such that incisor teeth were submerged (Fig. 1); the lesion was too extensive for surgical removal. The mass was pink, soft, and friable and had a surface composed of pinpoint nodules. Over time the condition worsened in severity, eventually affecting the buccal mucosa. In addition, numerous skin tags were evident on the chest, arms, and face of the monkey. The animal started to have trouble prehending food and lost weight; it was euthanized at 9 years of age. A necropsy revealed no evidence of metastases or other unusual findings.

Rhesus 3 and 4. Similar-looking oral lesions were identified in these two male monkeys after they both had resided at the NEPRC for 6 years. Rhesus 3 was born at the facility and rhesus 4 had been acquired as a 2-year-old. In the year prior to diagnosis, each was in a separate breeding cage with a harem of females; however, the two males had been housed together in a gang cage for the preceding 4 years. The nodules in rhesus 3 were observed while the monkey was being treated for a lingual laceration. They consisted of irregularly shaped, sessile, and contiguous masses on the rostral hard palate, abutting the upper incisor teeth (Fig. 2). Normal mucosal coloring was preserved on the granular and flattened surfaces. In rhesus 4, comparable masses were situated caudal both to the maxillary and mandibular central incisors (Fig. 3). Rhesus 4 also had a previous oral injury. Excisional biopsies were performed on both monkeys, and in rhesus 3 the 1.2-cm nodule that was removed regrew within 6 months. Nevertheless the oral lesions of both monkeys did not progress over a period of 7 years in the case of rhesus 3 or for 2 subsequent years prior to the euthana-

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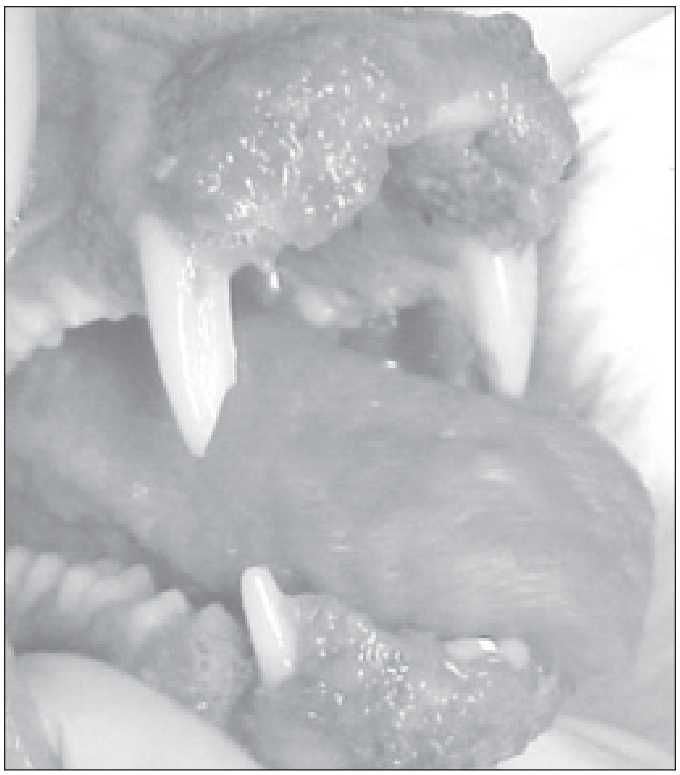

Figure 1. Photograph of oral mass found in rhesus 2, consisting of proliferative gingival tissue that extended between canine teeth of upper and lower jaws.

sia of rhesus 4. Other than traumatic incidents resulting from conspecific aggression, the medical histories of both rhesus macaques were unremarkable until the death of rhesus 4, which was associated with fatal fasting syndrome; the animals' research involvement was restricted to bone marrow aspirate collection. Females housed with these two males lacked any gross indication of oral or genital abnormalities.

Methods

Animal management. The Division of Comparative Medicine, MIT (Cambridge, Mass.), and the NEPRC (Southboro, Mass.) are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. The monkeys of this report received monkey chow (LabDiet 5038, PMI Nutrition International, Inc., St. Louis, Mo. or Diet 8714, Harlan Teklad, Madison, Wis.) along with daily food treats for environmental enrichment. Rhesus 1 had restricted access to water during short periods for training purposes as approved by the MIT Committee on Animal Care; otherwise water was available for the monkeys ad libitum. All animals underwent health monitoring on a regular basis and were determined to be negative for viral agents of concern in Old World nonhuman primates.

Light microscopy. Portions of each biopsy were placed in 10% neutral buffered formalin for histological examination. After dehydration and embedding in paraffin, 6-μm sections were cut and stained with hematoxylin and eosin.

Electron microscopy. For rhesus 3 and 4, fresh tissue was placed in 3% glutaraldehyde in 0.1 M phosphate buffer for electron microscopy. Epon-embedded sections were cut on an ultramicrotome and stained with uranyl acetate and lead citrate before viewing on a Jeol 1010 electron microscope (Peabody, Mass.). A paraffin-embedded section from rhesus 1 was prepared for electron microscopy.

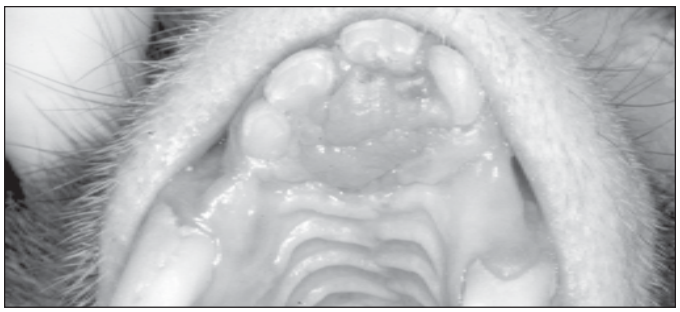

Figure 2. Photograph of sessile nodules located on hard palate behind incisors of rhesus 3.

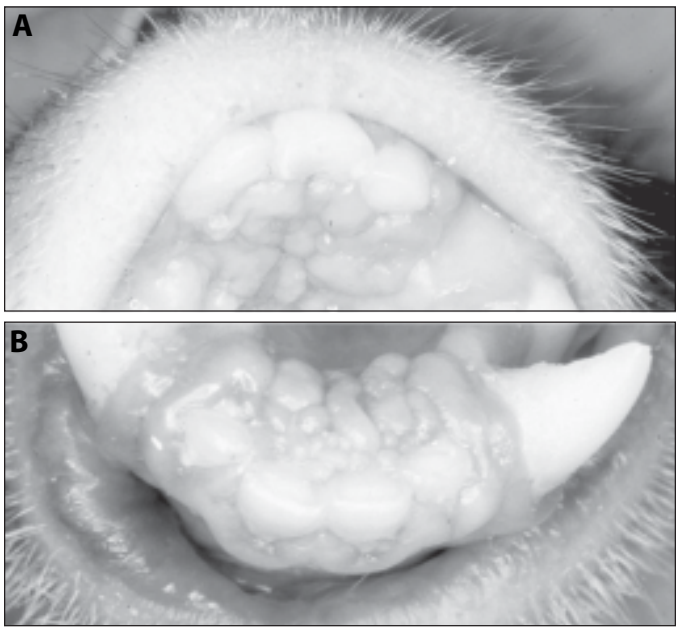

Figure 3. Photograph of irregular, coalescing nodules caudal to the maxillary (A) and mandibular (B) incisors in rhesus 4.

Polymerase chain reaction (PCR) amplification. A standard protocol for DNA isolation from paraffin-embedded tissues from rhesus 1 and 4 using proteinase K was followed (High Pure PCR Template Preparation Kit, Boehringer Mannheim Corp., Indianapolis, Ind., or QIAamp Tissue Kit, QIAGEN Inc., Santa Clarita, Calif.) PCR was performed with consensus primers MY09 and MY11 for the L1 gene of human papillomaviruses (14) as well as with E6 primers developed from rhesus monkey papillomavirus type 1 (6). DNA from human papillomavirus 16 served as a positive control for the human primer set. To confirm that the samples contained rhesus DNA, PCR amplification was run simultaneously using rhesus globin gene primers (F12 and G01).

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue sections from all four cases were assayed for papillomavirus genus-specific structural antigens using a rabbit polyclonal antibody to bovine papillomavirus (B580, DAKO Corp., Carpinteria, Calif.). Sections were rehydrated and exposed to heat-induced epitope retrieval as previously described

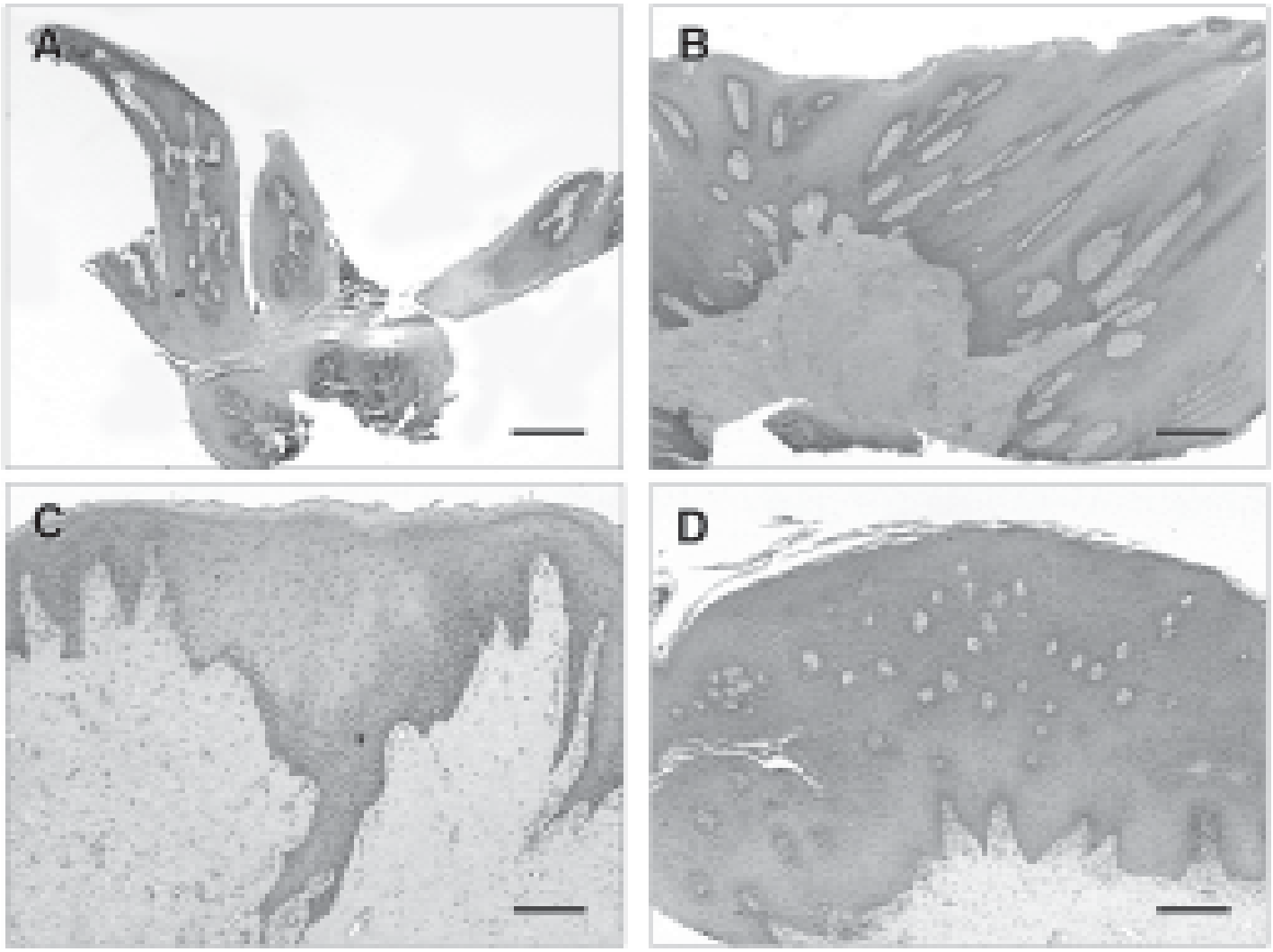

Figure 4. Histologic features of oral papillomas and papillary hyperplasia in rhesus macaques. H&E stain. (A) Solitary exophytic lesion from rhesus 1 characteristic of an oral filiform papilloma. Notice the long and ramificated connective tissue papillae and rete ridges of various shapes and lengths. Bar, 1000 μm. (B) Exophytic and proliferative tissue from rhesus 2 that was classified as a squamous papilloma. Notice the hyperplastic stratified squamous epithelium covering cores of fibrous connective tissue. Bar, 1000 μm. (C) Variably elongated rete ridges with occasional long, thin projections (pseudoepitheliomatous hyperplasia) and subjacent dense fibrous connective tissue from rhesus 3. Bar, 500 μm. (D) Papillary hyperplasia from rhesus 4. Notice the wavy surface with clefts, broad rete ridges, and subjacent fibrovascular connective tissue with mild inflammation. Bar, 600 μm.

(8), followed by a modified avidin–biotin technique. The antibody used has demonstrated cross-reaction with a broad array of mammalian papillomaviruses (11). Positive control tissues run in parallel were obtained commercially (DAKO).

Results

Microscopically, the focal exophytic oral mass of rhesus 1 appeared as a classic filiform papilloma with digitated papillary extensions, irregular epithelial rete ridges, and variably ramificated fibrovascular papillae cores (Fig. 4A). No koilocytes or intranuclear inclusion bodies were visible. The papilloma exhibited orthokeratotic hyperkeratosis and numerous mitotic figures. The proliferative tissue from rhesus 2 was composed of irregularly shaped, interconnected and variably sized papillary projections (Fig. 4B). The projections consisted of hyperplastic stratified squamous epithelium that was supported by fine stroma of fibroconnective tissue. There was marked intercellular edema in the epidermis. Scattered throughout the tissue were infiltrates of neutrophils and fewer lymphocytes. The morphologic diagnosis for rhesus 2 was squamous papilloma. Viral inclusions or koilocytes were not identified.

Samples from rhesus 3 (Fig. 4C) and 4 (Fig. 4D) were characterized by irregular, wavy surfaces with clefts, mild parakeratosis, predominance of large eosinophilic keratinocytes, and broad and irregular rete ridges with occasional long, thin projections (pseudoepitheliomatous hyperplasia). Mild-to-moderate, multifocal mixed inflammation of the subjacent fibrovascular connective tissue was present. The sections were compatible with a diagnosis of papillary hyperplasia (5, 7).

Electron microscopy did not identify viral particles in the sections chosen. No PCR product was amplified from the two rhesus samples tested using the human or rhesus papillomavirus primer pairs, but rhesus DNA was confirmed to be present. Immunohistochemistry did not demonstrate papillomavirus genus-specific antigens in the four rhesus samples while the control slide was positive.

Discussion

The rhesus monkeys of this report had oral masses of three distinct morphotypes: the first, a filiform papilloma (rhesus 1); the second, a more sessile but progressive squamous papilloma (rhesus 2); and the third characterized by irregular nodules that were consistent histologically with papillary hyperplasia (rhesus 3 and 4). Such lesions apparently are uncommon in macaque species and were of no clinical significance except in the case of rhesus 2. However these lesions are of comparative medicine relevance as each type is also diagnosed by human oral pathologists using light microscopy. A primary topic of interest relates to potential etiologies of the monkey tumors.

Certain histological hallmarks of papillomavirus infection (intranuclear inclusion bodies, koilocytes) were lacking in the two oral papillomas of this report, as well as in the proliferations identified as papillary hyperplasia; however, this absence does not preclude viral involvement. Our attempts to demonstrate papillomavirus by using electron microscopy, PCR and immunohistochemistry were also unsuccessful. Similarly an early review of papillomavirus infections in nonhuman primates showed that immunohistochemistry, in situ hybridization, and electron microscopy often failed to substantiate papillomavirus in suspect lesions (10), and cases of oral papillary tumors in humans likewise often are negative when assayed for virus (2, 4, 7, 12). Sampling error could be an influential factor in the present study. Also the PCR primers used could have been inappropriate in the event of a novel papillomavirus type, and strategies to identify papillomavirus antigens using immunohistochemistry are only successful in productive infections. In a recent study that examined cervical and vaginal neoplasms in cynomolgus monkeys by using immunohistochemistry with three different papillomavirus antibodies, the two mouse monoclonal antibodies did not reveal any additional positive cases when the lesions were papillomas compared to the rabbit polyclonal antibody against bovine papillomavirus (15). Therefore the similar rabbit polyclonal antibody chosen for this study, as in many others (11), would be likely to identify virus if present. In addition, sections from all four rhesus cases were stained with the three papillomavirus antibodies mentioned above and found to be negative (data not shown). Nevertheless, more than ten types of papillomaviruses have been classified from rhesus monkeys by using molecular techniques, albeit all of the samples for that work were obtained from reproductive tracts (1). The four monkeys of the present report, as well as all cohorts, were examined for genital lesions without any being suggested clinically.

Oral masses in humans are often attributed to persistent, low-grade trauma, and such chronic irritation could have played a role in the monkey tumors. For example, filiform papillomas like the one in rhesus 1 can develop in children that use their tongues to play with orthodontic appliances. Papillary hyperplasia that is comparable microscopically with the nodules in rhesus 3 and 4 occurs in humans as a result of poorly fitting dentures or unknown reasons (5, 7). Rhesus 4 had a longstanding tongue and incisor tooth injury, and rhesus 3 was undergoing treatment for a lingual laceration, raising the possibility of prior unrecognized incidents of oral trauma. Trauma, sucking on foreign objects, and malocclusion could all contribute to papillary hyperplasia in caged macaques. Although rhesus 3 and 4 lived in the same group enclosure for 4 years, there was no record of aggression specifically between them that might have allowed transmission of an infectious agent.

Differential diagnoses for the oral papilloma of rhesus 2 would include multiple hamartoma syndrome or Cowden's disease (2). The latter is a cancer-related, inherited condition in humans that usually involves pinhead-sized oral nodules that may coalesce. The tongue, lips, gingivae, and buccal mucosa can be affected. Skin tags, as were found on rhesus 2, or other skin lesions are a constant feature of multiple hamartoma syndrome. As far as was known, the sire and dam of rhesus 2 were unaffected by oral, dermal or neoplastic conditions. Another presentation for the lesions of rhesus 2 in humans would be related to immunosuppression; yet there was no reason to consider that the immune system of rhesus 2 was compromised. Finally gingival fibromatosis of rhesus macaques affects areas similar to the lesions of rhesus 2, and can also bury teeth in proliferative tissue, but it combines fibrous hyperplasia with a fairly normal stratified squamous epithelium (9). The progressive, even aggressive, nature of the papilloma found in rhesus 2 made it distinct from the three other cases described.

Oral papillomas resulting from papillomaviral infection in other animal species, especially dogs and rabbits, have been studied for a long time. In humans as well, a proportion of oral papillomas have been ascribed to human papillomavirus. Some human cases of papillary hyperplasia have been positive for papillomavirus (7), although trauma is considered the primary predisposing factor. While circumstances leading to the oral lesions of the four macaques documented here remain elusive and may involve nonviral factors, evidence of species-specific genital papillomaviruses in other rhesus macaques (1, 6) makes oral infection at least plausible. Additional cases of oral tumors in rhesus macaques may help to elucidate any causal relationship. Regardless, oral papillomas and papilliform lesions appear to be rare entities in the species, and in some instances they have no clinical impact.

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