Frequency and Distribution of Papillomavirus Structural Antigens in Verrucae, Multiple Papillomas, and Condylomata of the Oral Cavity

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Sixty-seven proliferous squamous epithelial lesions of the oral cavity were examined for the presence of human papillomavirus whole (structural) virion antigens by a peroxidase-antiperoxidase technique having immunospecificity against genus-specific (common) antigens of the papillomaviruses. A positive reaction for papillomavirus genus specific antigens was found in 18 of 29 verrucae, 2 of 5 multiple papillomas, and 3 of 5 condylomata; common antigens were not detected in 28 keratoacanthomas. The positive reaction was invariably intranuclear in cells having a focal or diffuse distribution in the superficial epithelium. This study shows that a variety of squamous epithelial lesions of the mucosa are associated with human papillomaviruses and suggests that these viruses may play an important role in the etiology of some cases of squamous hyperplasia of the oral cavity. (Am J Pathol 1982, 107:212–218)

FOR YEARS, circumstantial evidence supported the idea that most cutaneous warts[1,2] and some mucosal papillomas[2,3] were caused by a single type of human papillomavirus (HPV). The human wart virus has never been well-characterized by the standard methods of virology, and even today its oncogenic potential remains largely unknown for several reasons: HPV cannot be grown and tested in tissue culture,[4] and, like other papillomaviruses, it is highly species-specific[5,6] (transmission studies cannot be carried out in animal models). Recent advances in molecular virology, however, circumvented some of the difficulties of working with the papillomaviruses, resulting in a number of reports[7–9] indicating a remarkable plurality for HPV. The different types of HPV have little or no polynucleotide sequence homology, and there is no cross-reaction between virion surface antigens.[7] Six types of HPV (HPV-1 through HPV-5 and HPV-7) have been identified in a variety of proliferative lesions of the skin, and at least one type (HPV-6) was found in condylomata of the female genital tract. In fact, the type of papillomavirus appears to determine, in part, the clinical and pathologic appearance, location, and natural fate of cutaneous warts and perhaps some mucosal papillomas (Table 1).

Many different clinical types of papillomas (focal epithelial hyperplasia, verruca vulgaris and plana, multiple and single papillomas, condyloma accuminatum, laryngeal papillomas, and other papillomatous lesions) occur in the oral cavity. Although papovavirus-like particles have occasionally been observed by electron-microscopic examination in most of these lesions[2,3,10–17] papillomavirus has only specifically been identified by immunologic and molecular virologic techniques in laryngeal papillomas.[17–19] In this study we examined selected papillomatous lesions of the oral cavity with the same immunologic techniques used to identify papillomavirus genus-specific (common) antigens in laryn-

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Table 1—Types of Human Papillomavirus[9]

Virus	Clinical association
HPV-1	Plantar wart
HPV-2	Common wart
HPV-3	Flat warts
HPV-4	Plantar and common warts
HPV-5	Pityriasis-like lesions in epidermodysplasia verruciformis (EV) that may undergo malignant transformation
HPV-6	Anogenital warts
HPV-7	Common warts (butchers)

Adapted from Howley.[9]

geal papillomas[17-19] and genital tract condylomata[20] and now report on the prevalence of HPV in verrucae, condylomata, and multiple papillomas.

Materials and Methods

Papillomas

Squamous papillomas of the oral cavity selected for this study were accessioned in the files of the Armed Forces Institute of Pathology (AFIP), Washington, DC. Specifically, we wanted to examine verrucae, multiple papillomas, and condylomata for the presence of papillomavirus antigens, since papillomavirus-like particles have been seen in these lesions by electron microscopy; keratoacanthomas were examined because of their usual history of "wart-like" spontaneous regression. Verrucae and papillomas were retrieved from the files by location (oral cavity) and title (papillomas, squamous papillomas, verruca vulgaris, condyloma accuminatum, keratoacanthoma, florid papillomatosis, papillomatosis, verrucous hyperplasia).

After hematoxylin and eosin (H&E) sections from each papillomatous lesion were reviewed, at least 5 consecutive 4-μ-thick sections from paraffin blocks containing formalin-fixed tissue were air-dried on glass and stored at room temperature until ready for use.

Verrucae

Twenty-nine patients (25 male, 4 female) with oral cavity verrucae were included in this study. These verrucae had the following distribution: upper lip, 7; lower lip, 12; tongue, 4; alveolar mucosa, 2; hard palate, 2; buccal mucosa, 1; and floor of mouth, 1. The youngest patient was 8 and the oldest 61, but the majority were 20–30 years of age.

Condylomata

Five patients (all male) of condylomata were accepted for this study; 4 were on the tongue, and 1 was on the soft palate. Their ages were 23, 27, 31, 38, and 63. One patient had concurrent condyloma of the anal mucosa.

Multiple Papillomas

Five patients (4 male, 1 female) with multiple papillomas were included in this study. These lesions had the following distribution: 1) maxillary gingivae; 2) right and left commissures; 3) floor of mouth and tongue; 4) frenulum and mandibular gingivae; and 5) soft palate and left lateral tongue. The 4 males were 18, 24, 25, and 28 years of age; the female was 15. None of these patients stated in the record that they had other verrucous lesions.

Keratoacanthomas

Twenty-eight cases (27 male, 1 female) with keratoacanthomas were accepted for this study: 27 were located on the upper lip, and 1 was located on the lower lip. The youngest patient was 19, and the oldest was 74; the remaining patients were evenly distributed between these age limits. There was no mention of concomitant verruca at the time of removal of the keratoacanthomas.

Peroxidase-Antiperoxidase (PAP) Staining of Papillomas

Antiserums

Purified bovine papillomavirus type 1 (BPV-1) virions, previously characterized,[21] were extracted from a single naturally occurring bovine fibropapilloma. BPV-1 protein was prepared as previously described,[17,20,22] adjusted to 0.24 M 2-mercaptoethanol 1% SDS, heated to 68 C for 2 minutes, diluted with 3 volumes of saline, and mixed to an equal volume of Freund's complete adjuvant. A varying concentration of virus protein (480, 240, and 120 μg) was inoculated subcutaneously into a rabbit on Days 0, 15, and 28, respectively; and the animal bled on Day 38. This hyperimmune serum (BPV-1 [SDS]) is reactive by PAP at a 1:100 dilution with papillomavirus positive (by electron microscopy) cutaneous warts and mucosal papillomas from all species tested (human, cattle, dogs, rabbits, deer and horses) but does not react with virus-negative papillomas (data not shown); it has the same specificity but a higher titer for the papillomavirus genus-specific antigen as

an antiserum (HPV [SDS]) prepared against detergent-disrupted, heat-aggregated pooled plantar wart virus as previously described.[22]

PAP Staining

Sections of papillomas were deparaffinized, dehydrated, and washed with phosphate-buffered saline (PBS). After quenching of endogenous peroxidase activity with 0.5% hydrogen peroxide in methanol and a 30-minute incubation with 20% normal goat serum to reduce nonspecific staining, the sections were incubated with hyperimmune BPV-1 (SDS) serum at a dilution of 1:100 for 1 hour. The secondary antiserum was goat anti-rabbit immunoglobulin diluted 1:20 and left on the sections for 30 minutes. This was followed by a 30-minute incubation with a rabbit PAP complex diluted 1:50. The reaction was developed by the addition of 0.05%, 3,3′-diaminobenzidine tetrachloride and 0.01% hydrogen peroxide in 0.05 M-tris buffer (pH 7.6) for 5–8 minutes. Sections were then counterstained with hematoxylin, dehydrated, cleared, and mounted. The reactions and all washings performed between incubations were with PBS (pH 7.4).

The specificity of the BPV-1 (SDS) antiserum in the immunocytochemical reaction was verified by the control studies that were performed. A variety of normal mucosal tissues (cervix, foreskin, larynx, and gingivae) and brain tissue infected with herpes simplex virus Type 2 were reacted with the BPV-1 (SDS) antiserum; these all failed to show a positive reaction with the antiserum (negative control). In contrast, sections of virus-positive (by electron microscopy) human plantar warts (Figure 1) and canine oral papillomas run simultaneously with sections of oral cavity papillomas reacted positively with the BPV-1 (SDS) antiserum (positive controls). Negative controls performed simultaneously with the oral cavity papillomas under study consisted of serial sections incubated with normal rabbit serum and rabbit type-specific antiserum prepared against intact but pooled BPV-1 and BPV-2 virions.

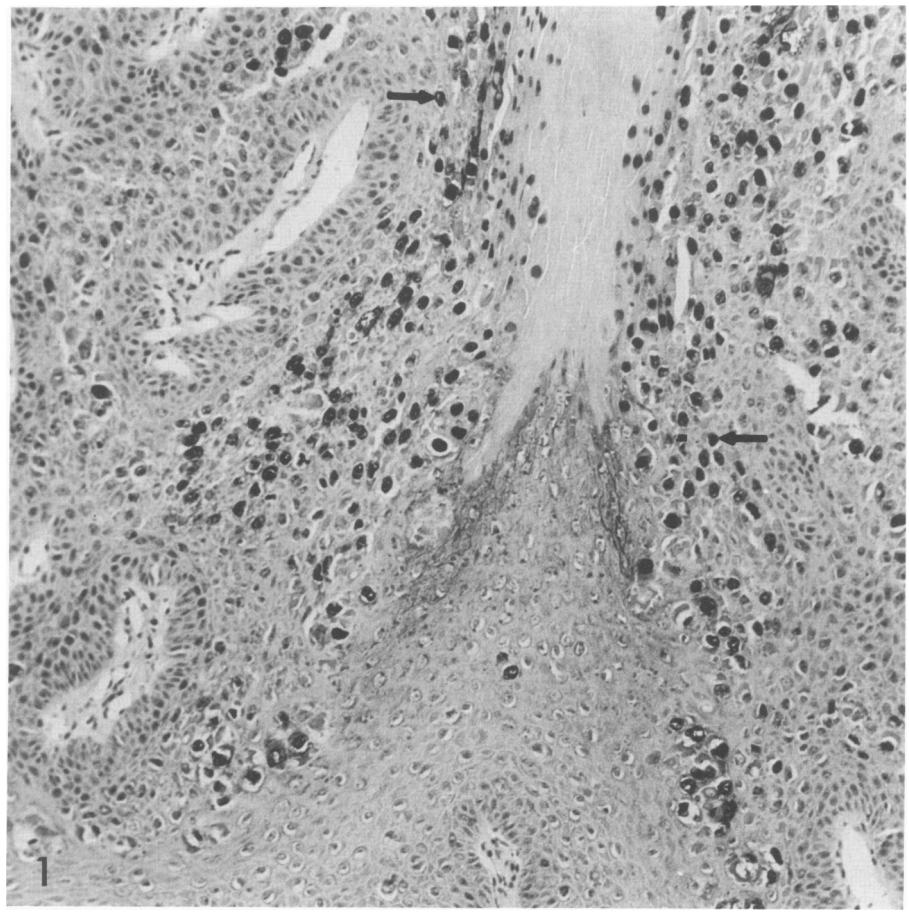

Figure 1—Plantar wart used as positive control for PAP reactions. HPV common antigens (brown precipitate, *arrows*) are seen in nuclei of prickle and granular cells before the latter undergo heratinization. (PAP and hematoxylin, ×197)

Table 2—Detection of Papillomavirus Common Antigens in Proliferative Squamous Epithelial Lesions of the Oral Cavity

	Anti-BPV-1 (SDS)	
Type of lesion	No. +	No. –
Verrucae		
Upper lip	7	0
Lower lip	8	4
Other	3	7
Multiple Oral Papillomas	2	3
Condylomata	3	2
Keratoacanthomas	0	28

Results

Anti-BPV-1 (SDS) was reactive with 18 of 29 verrucae, 3 of 5 condylomata, and 2 of 5 multiple papillomas; it did not react with any of the 28 keratoacanthomas (Table 2). The PAP stain (brown precipitate) was always localized to nuclei of squamous cells in the upper third of the epithelium (Figures 2–4); traces of nonspecific PAP staining was frequently seen in the cytoplasm but not in nuclei. In verrucae and condylomata, the intranuclear PAP staining pattern resembled that of a "raisin" and was most often seen in vacuolated (koilocytotic) cells with prominent keratohyalin granules. In the multiple papillomas, the PAP stain was usually seen in small clusters of cigar-shaped nuclei as well as nuclei of koilocytotic cells in the outermost layer of squamous epithelial cells.

Verrucae

All verrucae (7) of the upper lip and 8 of 12 from the lower lip were positive by PAP staining. Three of 7 verrucae from other locations (buccal mucosa, hard palate, and maxillary gingivae) in the oral cavity were also positive (Figure 2) for genus-specific antigen(s). Of the 6 patients with concomitant cutaneous verrucae, 3 had oral cavity verrucae that were positive by PAP.

Condylomata

The 3 (of 5) PAP-positive condylomata (Figure 3) were located on the tongue. The 1 patient with a con-

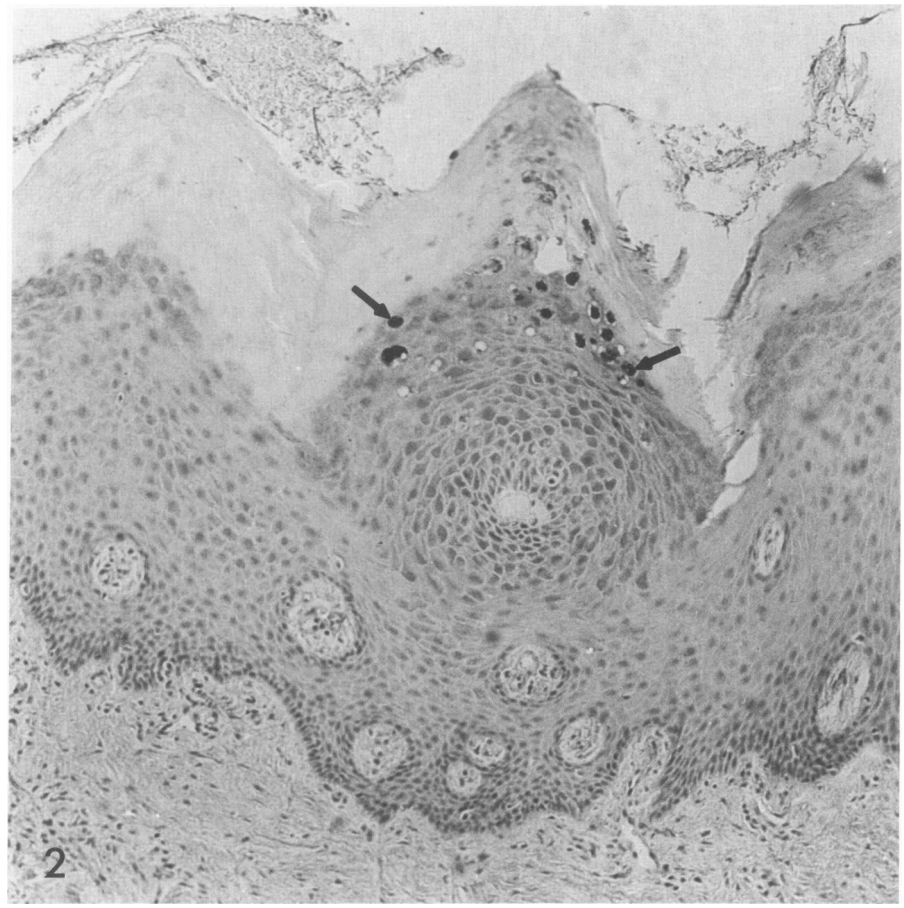

Figure 2—Verruca from hard palate. Papillomavirus structural antigens are identified by positive PAP reaction (*arrows*) in outer squamous epithelial cells of only 1 papillary frond in this photomicrograph. (PAP and hematoxylin, ×197)

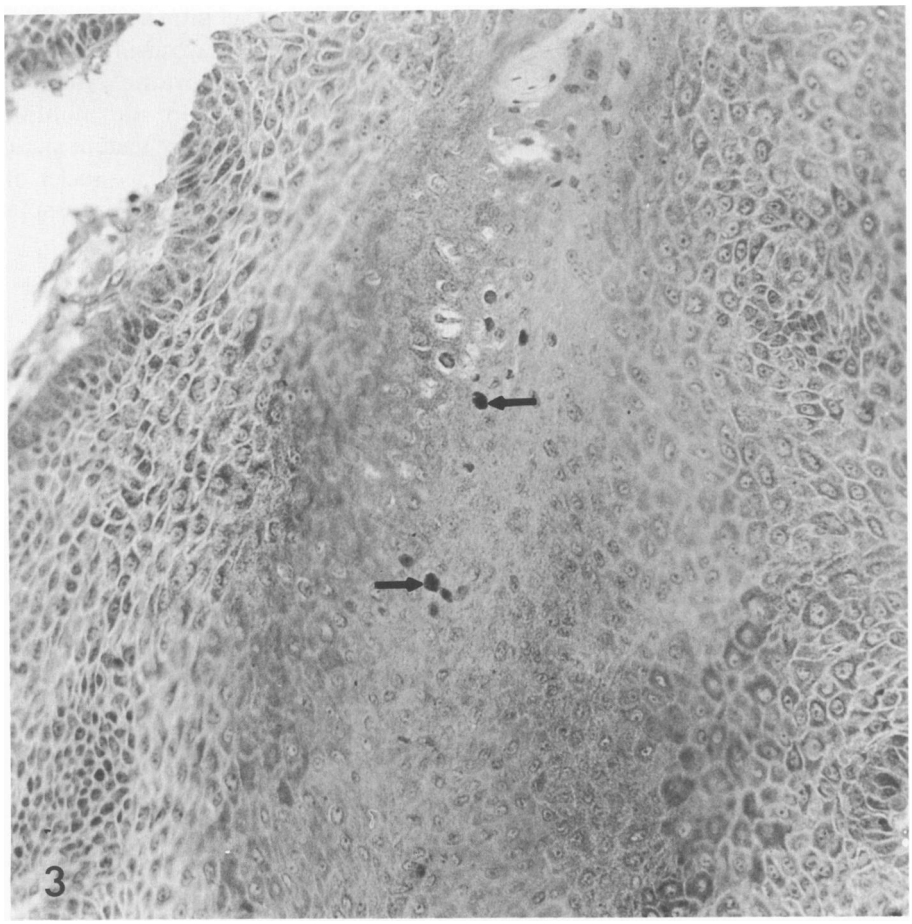

Figure 3—Multiple papilloma from maxillary gingiva. Tangential section shows several clusters of superficial squamous cells with nuclei that are PAP-positive (*arrows*) for papillomavirus. (PAP and hematoxylin, ×197)

comitant anal-genital condyloma had a PAP-positive oral cavity condyloma.

Multiple papillomas

The 2 (of 5) PAP-positive multiple papillomas (Figure 4) were from the soft palate and maxillary gingivae and lateral tongue, respectively.

Keratoacanthomas

All (28) keratoacanthomas were PAP-negative.

Discussion

The papillomavirus genus, together with the polyomavirus genus, constitutes the family Papovaviridae.[23] A previous study showed that all viruses of the same genus possess common whole (structural) virus antigens that can be detected in nuclei of infected cells by antiserum prepared against disrupted but not intact virions of any member of the genus.[22] The antiserum used by us and others to identify papillomavirus genus-specific antigens in formalin-fixed paraffin-embedded verrucae, condylomata, and multiple papillomas (Table 2) and laryngeal papillomas[24] of the oral cavity and cervical dysplasias and vulvar condylomata of the female genital tract[20] was prepared from detergent-disrupted bovine papillomavirus Type 1 (BPV-1 [SDS]) obtained from cutaneous fibropapillomas of cattle. Besides being a readily available source of large quantities of papillomavirus, the use of purified BPV-1 (SDS) as antigen provides an antiserum that would be less likely to give false reactions with endogenous intranuclear proteins and DNA in human tissue than an antiserum derived from HPV.

Since papillomaviruses do not appear to exist in nature as passenger viruses, identification of HPV in a cutaneous wart or mucosal papilloma in all likelihood establishes the etiology of the lesion. Failure to detect HPV, however, does not necessarily exclude it as the etiologic agent. In this study papillomavirus common antigens were identified in 62% (18 of 29) of

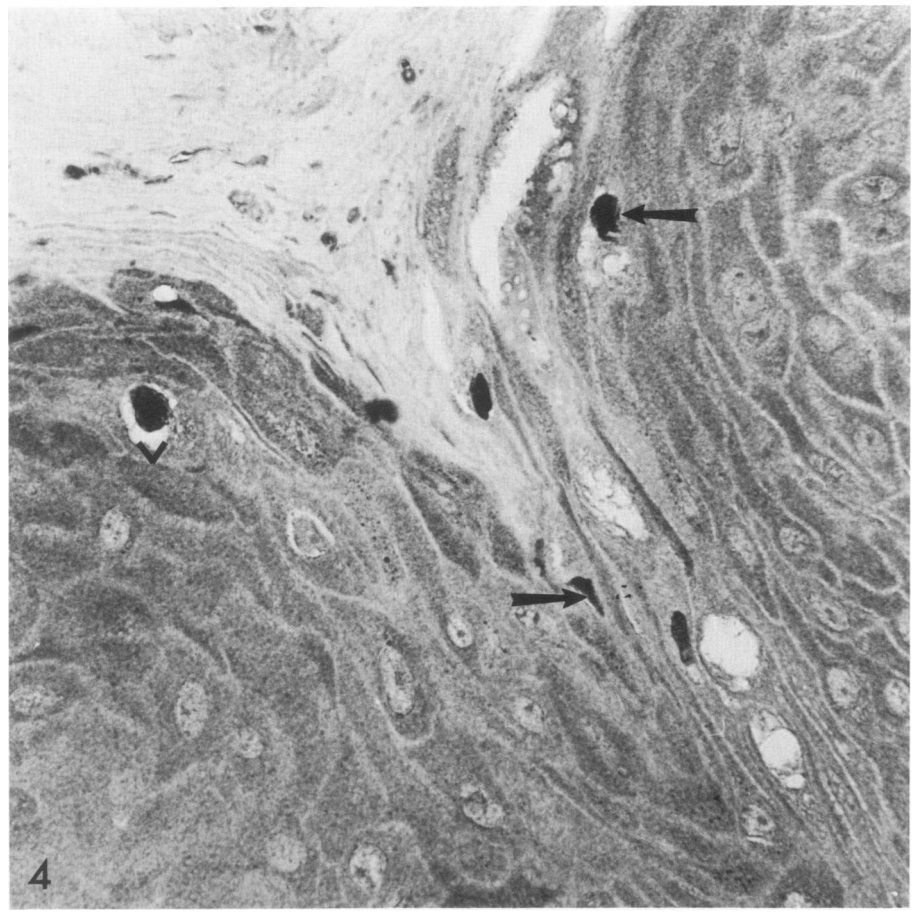

Figure 4—Condyloma from tongue. Positive intranuclear reaction (*arrows*) for HPV common antigens can be identified in koilocytotic cells (vacuolated [*V*] cells) as well as in more superficial, flattened squamous epithelial cells. (PAP and hematoxylin, ×450)

the verrucae, 60% (3 of 5) of the condylomata, 40% (2 of 5) of the multiple papillomas, and none (0 to 28) of the keratoacanthomas of the oral cavity. This is consistent with a detection rate of about 50% of papillomavirus shared antigens in cutaneous warts,[25] laryngeal papillomas,[17,18] and condylomata of the male[25] and female genital tract.[20] That this may be due to either sampling error or periodic expression of virus antigens by infected cells is suggested by a recent study of 102 recurrent laryngeal papillomas surgically excised from 35 patients.[17] When only 1 laryngeal papilloma was available for staining, the detection rate for HPV was 48%. However, when 4 or more consecutive biopsies from the same patient were available for study, at least 1 of the biopsies was always positive for HPV. Although there was only 1 sample to test on each patient in the present study, we provided direct evidence of a papillomavirus association for the 23 oral cavity papillomas containing structural viral antigens (Table 2).

Typing of HPV in tissue available for retrospective studies such as ours can only be accomplished by staining with type-specific antiserum, which is currently not available. By using genus-specific antiserum, however, HPV can be identified regardless of type, and this information can then be used for prospective studies. Patients with representative lesions can be located and fresh samples obtained for characterization of papillomavirus polynucleotide sequences by restriction endonuclease cleavage patterns and molecular hybridization studies necessary to identify new types of HPV.[7] We are using this approach to study oral cavity lesions, since it is likely that some will be caused by new types of HPV. Various studies have shown that most types of papillomavirus have a tropism for either the mucosa or skin, rarely both.[1-3,5,6] Evidence that this might also be true in man comes from the fact that HPV-6 has only been found in mucosal lesions.[7-9] Since we know that at least four different clinical types of oral papillomas are associated with HPV, determining whether the differences in appearance of the lesions are due to new types of HPV, the site of infection (ie, the larynx) or both will be of interest.

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