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NOTES

Characterization of Human Papillomavirus Type 13 from Focal Epithelial Hyperplasia Heck Lesions

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Focal epithelial hyperplasia Heck lesions of a Turkish patient were shown to contain papillomavirus-specific DNA, which was molecularly cloned into bacteriophage lambda. It proved to be related to human papillomavirus (HPV) type 6 DNA and HPV type 11 DNA. Reassociation kinetics revealed a cross-hybridization of 4 and 3%, respectively. There was no cross-reactivity with HPV type 1, 2, 3, 4, 5, 8, or 10. This papillomavirus type will be referred to as HPV type 13. The DNA was characterized by cleavage with several restriction enzymes, and the cleavage sites were physically mapped. Papules from two additional cases of Morbus Heck contained HPV type 13 DNA as shown by Southern blot hybridization and by the characteristic cleavage patterns. This may indicate that HPV type 13 is more frequently associated with focal epithelial hyperplasia Heck than are other HPV types.

Focal epithelial hyperplasia Heck (FEH) was first described in American Indians (1). The disease also appears in other races (13; for a review, see reference 24) but seems to be very rare in Caucasians. It occurs mainly in children and young adults, with frequent manifestation within the same family. Multiple, slightly elevated papules appear on the red surfaces of the lips and on the labial and buccal mucosa, with a tendency to confluence. The surface of the papules is smooth, with or without weblike markings, and of the same color as the adjoining normal mucosa. The lesions may persist for several years but do not become malignant and finally tend to spontaneous remission.

Papillomavirus-like particles were repeatedly detected in Morbus Heck lesions, which points to a viral etiology (8, 9, 12, 16, 19, 25). In one case the virus was typed as human papillomavirus (HPV) type 1 (HPV1) (17). Rather often, however, it was impossible to isolate virus particles (13, 23) or even to demonstrate virus-specific DNA (Pfister, unpublished data). This may be due to very small amounts of viral fingerprints, which are below test sensitivity, or to the use of inappropriate probes, in view of the great heterogeneity of HPVs (for a review, see reference 11).

We examined lesions from a 13-year-old Turkish girl with typical FEH. She had been suffering for the past 3 years from multiple skin-colored papules and plaques on the red surfaces of the upper and lower lips, the corners of the mouth, and the buccal mucosa (Fig. 1a). The epithelium of the oral mucosa showed a strong acanthosis with elongated rete ridges, a significant papillomatosis, and a continuous parakeratosis. The rete ridges were bent inward at both margins and thereby appear to point radially to the center. Large vacuolated cells with deeply basophilic nuclei were found focally in both the lower- and the upper-stratum malpighii (Fig. 1b and c).

DNA was extracted from the lesions by phenol treatment (26), and 500 μg of the DNA was subjected to CsCl-ethidium bromide equilibrium centrifugation (20). Gradient fractions with a density between 1,597 and 1,600 g/cm^3 were tested for papillomavirus-specific DNA by agarose gel electrophoresis. Ethidium bromide staining revealed one band comigrating with supercoiled HPV1 DNA, which corresponds to a molecular weight of 5×10^6. For virus classification FEH DNA was hybridized to a set of HPV reference DNAs. HPV1 DNA was isolated from virus particles as described previously (5). HPV2, HPV5, and HPV10 DNAs were prepared

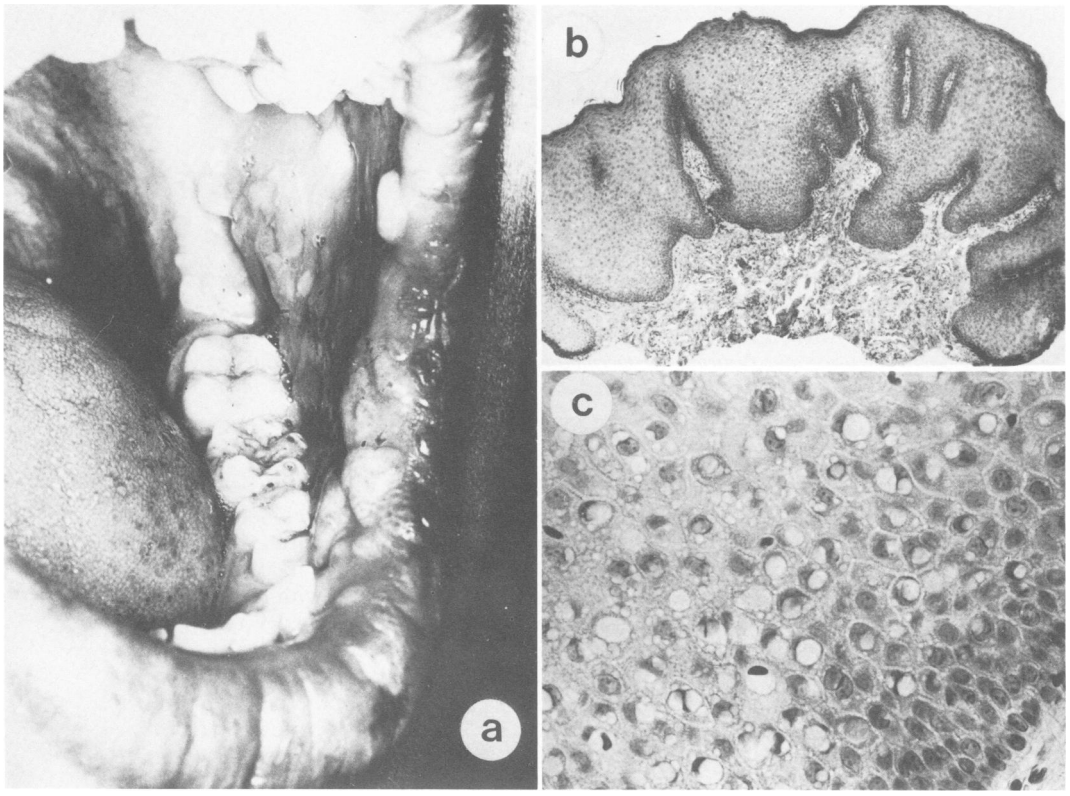

FIG. 1. FEH lesions. (a) Papules on the lips and buccal mucosa of the patient. (b) Histology of the papules at low magnification showing papillomatosis and acanthosis with elongated rete ridges pointing radially toward the center. (c) Vacuolated keratinocytes in the lower-stratum malpighii with deeply basophilic, partially pycnotic nuclei.

from common warts, epidermodysplasia verruciformis lesions, and flat warts, respectively, and were characterized by cleavage with several restriction endonucleases. HPV10 DNA cross-hybridizes with HPV3 DNA under stringent conditions (G. Orth, personal communication) and was used as a reference for both HPV3 and HPV10. Cloned DNAs were available for HPV4 (10), HPV6 (3), HPV8 (18), and HPV11 (4). FEH DNA was eluted from the gel, radioactively labeled with ^{32}P (18), and hybridized to filter-bound DNA of FEH, HPV4, HPV5, and HPV8. The probe reacted with homologous DNA but with none of the HPV DNAs. In a second set of experiments FEH DNA was cleaved with *Bam*HI, separated electrophoretically, blotted to nitrocellulose filters, and hybridized with ^{32}P-labeled HPV1, HPV2, HPV6, HPV10, and HPV11 DNA. All probes were negative except for HPV6 and HPV11. Both DNAs led to weak signals, however, when compared with equal concentrations of the homologous DNAs.

The FEH isolate was cloned in bacteriophage lambda L47 for further classification and characterization. It had become clear that the viral DNA had more than one *Bam*HI cleavage site. To enable cloning of the entire genome, total DNA from the FEH biopsies was only partially digested with this enzyme. The reaction was stopped by addition of EDTA, and after the addition of lambda L47 DNA, which was completely cleaved with *Bam*HI, the mixture was extracted with chloroform-isoamylalcohol. Ligation, in vitro packaging, identification of HPV DNA-containing phages, and phage amplification were by published protocols (4). We obtained different clones, which harbored one, two, or three viral DNA fragments, and chose one clone with all three fragments (Fig. 2). A comparison of the cleavage patterns of cloned viral DNA and of viral DNA from biopsy material confirmed that there were no major rearrangements during the cloning procedure.

FEH papillomavirus (FEH-PV) DNA was tested for sensitivity against a number of restriction enzymes. There were no cleavage sites with *Eco*RI, *Hin*dIII, *Sal*I, *Sac*I, and *Bcl*I. The cleavage sites of *Bam*HI, *Hin*dII, *Hpa*I, *Pvu*II, and

*Sph*I were physically mapped by double cleavages of biopsy DNA and by *Bam*HI/enzyme X double cleavages of cloned DNA (Fig. 3).

The extent of homology between FEH-PV and HPV6 and HPV11 was determined by reassociation kinetics according to the method of Sharp et al. (21). Briefly, unlabeled and labeled DNAs were denatured by boiling for 15 min, chilled in ice, and reassociated at 68°C in 0.5 M NaCl–17 mM EDTA–0.1% Sarkosyl. Samples were taken at various times, and single- and double-stranded DNAs were separated by hydroxyapatite chromatography. Unlabeled DNA (100 ng) from the three virus types was used to accelerate the hybridization of 100 pg of ^{32}P-labeled FEH-PV DNA. FEH-PV showed 4% cross-hybridization with HPV6 and 3% with HPV11. Corresponding values were obtained with labeled HPV6 or HPV11 DNA and unlabeled FEH-PV DNA.

According to these data, the FEH isolate represents an independent virus type with less than 50% cross-hybridization (2) and a totally different restriction enzyme cleavage map. In view of the weak cross-hybridization with HPV6 and HPV11, however, it is interesting to note that both virus types are associated with mucosal tumors, too: HPV6 is associated with condylomata acuminata (7), and HPV11 is associated with laryngeal papillomas (4).

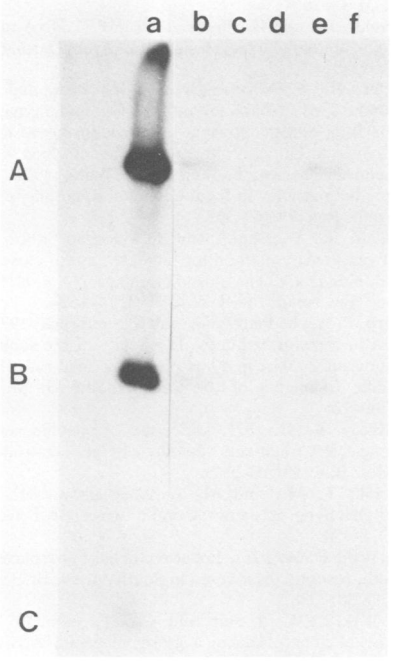

FIG. 2. Test for papillomavirus-specific sequences in *Bam*HI-cleaved DNA from five cases of FEH (lanes b–f) by hybridization with ^{32}P-labeled FEH-PV DNA. Two faint bands each are detectable in two preparations (lanes b and e); these bands comigrate with *Bam*HI fragments A and B of cloned FEH-PV DNA (lane a). Virus-specific DNA was demonstrated among cellular DNA according to the method of Southern (22).

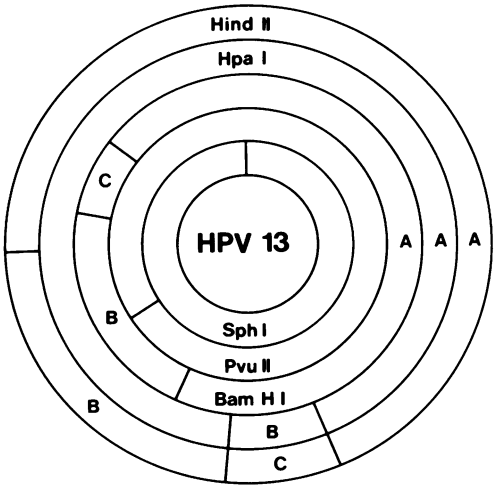

FIG. 3. Restriction enzyme cleavage map of FEH-PV DNA, which was later designated HPV13. Each circle represents the cleavage sites of one enzyme. Restriction endonucleases were purchased from Bethesda Research Laboratories and were used with the reaction buffers recommended by the manufacturer. Cellular DNA (10 μg) or cloned DNA (300 ng) was incubated at 37°C for 3 h with 10 or 1 U of enzyme, respectively. After ethanol precipitation, DNA cleavage products were suspended in electrophoresis buffer and applied to 1, 1.5, or 2% agarose gels. Electrophoresis was carried out at 40 V overnight, using a Tris-acetate buffer (pH 8.1).

As described above, there was no relationship detectable with HPV types 1, 2, 3, 4, 5, 8, and 10. HPV7 is isolated from skin warts of butchers and meat handlers (14, 15) and is not related to HPV6. HPV12 is derived from lesions of epidermodysplasia verruciformis patients and shows clear homology with HPV5 and HPV8 DNAs (G. Orth, personal communication). These data argue against a relationship between the FEH isolate and HPV7 or HPV12. We therefore propose to designate the FEH isolate as HPV13.

DNAs from five additional cases of FEH were tested for HPV13-specific sequences to assess the prevalence of the new isolate. The diagnosis of FEH was based on clinical and histological examinations. Southern blot hybridization of *Bam*HI-cleaved DNAs to cloned HPV13 DNA labeled with ^{32}P revealed two faint bands each in two cases. They comigrated with the two major *Bam*HI fragments of HPV13 DNA (Fig. 2), which indicates the presence of a closely related or identical virus type. The *Bam*HI fragment of 0.36×10^6 daltons is probably missed due to the

very low concentration of viral DNA. The three negative DNAs were also negative under relaxed hybridization conditions (Tm − 50°C).

The presence of HPV13 in three of six cases of FEH suggests that it is more regularly associated with this disease than are other HPV types. The concentration of viral DNA always was very low. In two cases viral DNA was only detected by Southern blot hybridization with HPV13 DNA, and the signals were very faint. They were not revealed earlier by hybridization with HPV6 under stringent or relaxed hybridization conditions (data not shown). The frequently negative outcome of the search for papillomaviruses in FEH becomes understandable from these results: on the one hand, there are only low amounts of viral DNA, and on the other hand, there are no appropriate probes available so far. In the three negative biopsies in this study, the amount of viral DNA might well have been below the test sensitivity. However, there also exists the possibility that other, not yet identified HPV types will be found in FEH lesions.

In view of the recent demonstration of HPV11 DNA in cervical carcinomas (6), 20 carcinomas from the genital region were tested for the presence of FEH-PV DNA and proved to be negative. This would be in line with the usually benign course of FEH. Nevertheless, head and neck tumors especially should be thoroughly tested for HPV13 sequences.

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