Detection of Mucosal Human Papillomavirus Types 6/11 in Cutaneous Lesions from Transplant Recipients

Chantal Soler,* Yvette Chardonnet,* Patrice Allibert,† Sylvie Euvrard,* Daniel Schmitt,* and Bernard Mandrand†
*INSERM U346 Affiliée CNRS, Pavillon R, Hôpital E. Herriot, Lyon, and †UMR103 CNRS/ BioMérieux, Ecole Normale Supérieure, Lyon, France

Transplant recipients develop multiple cutaneous lesions. We have identified human papillomavirus (HPV) DNA in these lesions using three different techniques, namely polymerase chain reaction (PCR), *in situ* hybridization, and Southern blotting. By PCR, HPV DNA was detected in 43 of 62 samples: warts, actinic keratoses, Bowen's disease, and squamous cell carcinomas. Surprisingly, HPV 6/11, usually associated with mucosa, were frequently found in benign, premalignant, and malignant cutaneous lesions (30/43 cases). Some of these biopsies were simultaneously tested by *in situ* hybridization and/or Southern blotting. By *in situ* hybridization, HPV 6/11 were identified in two warts and one squamous cell carcinoma among 29 tissue specimens tested. Of the three samples examined by Southern blotting, HPV 6/11 were detected in one squamous cell carcinoma. In patients from a control population cutaneous biopsies did not exhibit HPV types 6/11 except in Bowen's disease; HPV types 1 or 2 were mainly found in benign warts. These findings suggest that in transplant recipients, HPV can lose their specificity towards mucosa or cutaneous epithelium. The significance of the presence of HPV 6/11 in skin lesions remains unknown. Key words: HPV 6/11/skin lesions/renal transplant recipients/cardiac transplant recipients. *J Invest Dermatol 101:286–291, 1993*

Immunosuppressed patients such as transplant recipients develop numerous cutaneous lesions, profuse verrucosis, and carcinomas on sun-exposed areas [1,2]. In these benign and malignant lesions HPV DNA has been detected [3–6]. Several HPV types have been detected in such biopsies: HPV 1, 2, 3, 4, and 10 were frequent in skin warts [3,7], as in the general population; benign HPV 2 and 4 were also found in squamous cell carcinomas [4], oncogenic HPV 16 was seen in benign skin warts [7] and in periungual squamous cell carcinomas [8,9]. HPV 6/11, which usually infect benign genital lesions such as condylomas or benign laryngeal papillomas of the general population [10,11], have also been detected in perianal squamous cell carcinomas from transplant recipients [12]. This investigation reports the detection of mucosal HPV types 6/11 in different cutaneous lesions from transplant recipients using PCR and, in some specimens, *in situ* hybridization and Southern blotting.

MATERIALS AND METHODS

Biopsy Specimens Sixty-two cutaneous biopsies were excised from 22 renal and five cardiac transplant recipients with multiple lesions on sun-exposed areas (face, arm, hand, leg, top of back, and chest). Ten skin biopsies were taken from normal individuals with similar lesions, and six genital warts and five juvenile laryngeal papillomas were also tested.

Each biopsy was cut into two parts: one was snap frozen and stored at −20°C and the other was fixed in Bouin's or Baker's solution and embedded in paraffin. Four-micrometer frozen sections were layered on aminopropyltriethoxysilane (Aldrich Chemie, Strasbourg, France)-treated slides and fixed in acetone for *in situ* hybridization. One slide from each paraffin-embedded lesion was routinely stained with hematoxylin-eosin for histologic examination.

Polymerase Chain Reaction Studies Total cellular DNA was extracted from biopsies by phenol-chloroform-isoamyl alcohol and precipitated with ethanol. PCR was performed on cellular DNAs using a Perkin-Elmer Cetus DNA thermal cycler according to the procedure and precautions previously described [13], with primers chosen in E6–E7 region of HPV types 5, 6/11, 16, and 18 (Table I). The amplification protocol was annealing for 1 min at 55°C, extension for 1 min at 72°C, and denaturation for 1 min at 95°C, except for HPV 5, for which the annealing temperature was 58°C. Amplified products were detected on ethidium bromide-stained gels and Southern blots were hybridized with radiolabeled specific oligonucleotides (Table I).

To prevent artefactual results, cellular and plasmid DNAs were prepared in different rooms. Furthermore, some cellular DNAs used in these experiments were extracted in different years. Buffer and primers were submitted to UV for 3 h and aliquoted. Positive and negative controls were included in the reactions.

***In Situ* Hybridization** HPV 1a, 2a, 5, 6a, 11, 16, and 18 (provided by G. Orth, Paris and H. zur Hausen, Heidelberg) cloned in pBR322 plasmid were prepared and purified on cesium chloride gradients. Plasmid DNAs were biotinylated by nick translation using biotinylated 11-dUTP (Sigma, Saint Louis, MO). The sections were processed under stringent conditions at Tm-17°C and DNA-DNA hybrids were detected with a three-step procedure using streptavidin-alkaline phosphatase complex [14].

Southern Blot Ten micrograms of total cellular DNA from eight biopsy-specimens (three from transplant recipients and five from controls) were digested with Pst I (Boehringer, Mannheim, Germany) in buffer from the supplier and electrophoresed on a 1% agarose gel. The fragments were then transferred onto a Gene Screen Plus membrane (NEN, Boston, MA). Plasmid HPV types 1a, 2a, 5, 6a, 11a, 16, and 18 probes were labeled by nick translation using ^{32}P-dCTP (specific activity: 800 Ci/mmol; Amersham). Membranes were prehybridized in 10 ml of 50% deionized formamide, 1% sodium dodecyl sulfate (SDS), 1 M NaCl, 10% dextran sulfate, for 6 h at 42°C. One milliliter of solution containing about 5×10^6 cpm of labeled probe (specific activity: 8×10^7 cpm/μg DNA) and 100 μg of salmon sperm DNA were added after denaturation by heating in boiling water for 10 min and chilling on ice. Hybridization was carried out overnight at 42°C. The membranes were carefully washed successively in 2 × sodium saline citrate (1 × SSC: 0.15 M NaCl, 0.015 M sodium citrate), 2 × SSC plus 1% SDS,

Manuscript received June 24, 1992; accepted for publication May 3, 1993.
Reprint requests to: Dr. Y. Chardonnet, INSERM U346 Affiliée CNRS, Pavillon R, Hôpital E. Herriot, 69437 Lyon Cedex 03, France.
Abbreviation: dUTP, deoxy uridine triphosphate.

0022-202X/93/$06.00

Table I. Primers and Oligoprobes for HPV 5, 6, 11, 16, and 18 Chosen in E6-E7 Region

HPV	Localization	Sequences	Size of Fragments	Tm
Primers				
HPV 5	609–630	5′ TTCATAAGGTGAGGAACGCCTG 3′		
	797–897	3′ CGAAAGGTTGTCGATGACTGGC 5′	288 bp	
HPV6/11	514–533	5′ TACACTGCTGGACAACATGC 3′		
	797–815	3′ CACACAGGGTAGACGCGTG 5′	301 bp	
HPV 16	548–571	5′ CCCAGCTGTAATCATGCATGGAGA 3′		
	778–801	3′ ACCTTCTGGACAATTACCCGTGTG 5′	253 bp	
PVH 18	540–560	5′ CGACAGGAACGACTCCAACGA 3′		
	718–741	3′ TCAATTAGTAGTTGTAAATGGTCG 5′	201 bp	
Probes				
HPV 5	797–818	5′ CAACGAAAGGATCTCTTACAAA 3′		50.5°C
HPV 6/11	584–602	5′ GACCTGTTGCTGTGGATGT 3′		69°C
HPV16	700–717	5′ CCGGACAGAGCCCATTAC 3′		62.3°C
HPV18	601–620	5′ TAAGGCAACATTGCAAGACA 3′		59.2°C

and 0.1 × SSC solutions. They were further exposed to X-OMAT (Kodak) films for 1–14 d using cassettes with intensifying screens. Membranes were successively hybridized with the different HPV probes after dehybridization according to the manufacturer's instructions and control for the loss of radioactivity.

RESULTS

All biopsy specimens were examined after staining with hematoxylin-eosin and were classified according to their histologic criteria. Data comparing histologic diagnosis and results of experimental studies are shown in Table II.

Transplant Recipient Lesions PCR amplification of HPV types 5, 6/11, 16, and 18 was performed on skin lesions. Of the 62 samples, 43 (69%) were HPV DNA positive; 30 of 43 (69%) exhibited HPV 6/11 DNA, four of 11 (36%) benign vulgaris warts, seven of seven premalignant actinic keratoses, four of four premalignant Bowen's disease, and 15 of 21 (74%) squamous cell carcinomas (Table II).

Among 30 biopsies containing HPV 6/11 DNA by PCR (Table III), 12 samples (three warts, four actinic keratoses, three Bowen's disease, two squamous cell carcinomas) contained these HPV types; the other probes gave negative reactions. HPV 5 and HPV 6/11 were found in seven cases (one actinic keratosis, six squamous cell carcinomas). The other 11 samples contained HPV 6/11 and HPV 16, 18, and/or 5 (one wart, two actinic keratoses, one Bowen's disease, seven squamous cell carcinomas).

After HPV 6/11 amplification and hybridization of Southern blots with radioactive probes, bands of various intensities were seen, indicating that various amounts of HPV 6/11 DNA were present in these samples. Figure 1 shows an example of results obtained after amplification of squamous cell carcinoma DNAs. In samples containing more than one type of DNA, HPV 6/11 were predominant in benign and premalignant lesions, but in squamous cell carcinomas they were present in smaller amounts than in oncogenic types.

HPV 6/11 DNA was identified (Table III) not only in seven patients with single biopsies but also in seven other patients having several excised lesions. In this latter case, samples were taken at different periods, therefore excluding the possibility of contamination during DNA extraction. Furthermore, amplification without DNA or amplification of either MRC5 cell DNA or dermal DNA extracted from normal skin gave negative results in every case. Because HPV 6/11 were usually found in benign mucosal lesions, PCR amplifications and analyses of amplification products were repeated at least two or three times. Similar results were detected consistently.

Twenty-nine of the 62 biopsies (Table IV) were also screened for the presence of HPV DNA by *in situ* hybridization with HPV type 1, 2, 5, 6/11, 16, and 18 probes; 12 of 29 contained HPV DNA. The positive signal was always nuclear and was located in intermediate and upper epithelial cell layers. Furthermore, it was usually in foci of a few infected cells. HPV 6/11 were identified in three samples: two warts and one squamous cell carcinoma (Fig 2); these results were confirmed with PCR. In warts, HPV 6/11 were associated with HPV 1 and/or 2 and 16; in the squamous cell carcinoma, only HPV types 6/11 were identified. Three to five sections per sample were tested by *in situ* hybridization, which gave similar reactions.

Tissue sections were negative when the DNA probe was omitted or replaced by the pBR322 probe. HPV 16 and 18 probes were assayed with CaSki, SiHa, and HeLa cells containing, respectively, 600 copies of HPV 16, one to two copies of HPV 16, and 10–50 copies of HPV 18. CaSki and SiHa cells were positive only with HPV 16 probe, HeLa cells only with HPV 18 probe.

Comparing the results obtained by PCR and *in situ* hybridization

Table II. Frequency of HPV 6/11 Detected by PCR in Skin Lesions from Transplant Recipients and Normal Population[a]

	Transplant Recipients			Non-Transplant Recipients		
Lesions	Total	HPV[b] DNA +	HPV[c] 6/11 +	Total	HPV[b] DNA +	HPV[c] 6/11 +
Warts	18	11	4 (36%)	3	0	0
Keratoacanthomas	2	0	0			
Actinic keratoses	12	7	7 (100%)			
Bowen's disease	6	4	4 (36%)	3	2	2
Squamous cell carcinomas	24	21	15 (71%)	4	2	0
Total	62	43	30 (69%)	10	4	2
Controls						
Genital condylomas				6	6	6
Laryngeal papillomas				5	5	5

[a] PCR amplification of HPV types 5, 6/11, 16, and 18.
[b] HPV DNA +, number of lesions containing HPV DNA.
[c] HPV 6/11 +, number of lesions containing HPV types 6/11 DNA.

Table III. Detection of HPV 6/11 by PCR[a] in 30 Skin Lesions from Transplant Recipients

Patients	Lesions	Number	Localization	HPV Types 5	6/11	16	18
Renal transplant recipients							
A	Warts	1	Arm		+[b]		
B	Warts;	2	Finger		+		
	actinic keratosis	3	Neck		+		
		4	Finger	+	+		
	Squamous cell carcinomas	5	Back	+	+	+	
		6	Hand		+		
		7	Back		+		
		8	Back	+	+		
		9	Back	+	+		
		10	Back	+	+		
		11	Neck	+	+		
C	Warts	12	Cheek		+		+
D	Warts	13	Forehead		+		
E	Actinic keratosis;	14	Nose		+		
	squamous cell carcinoma	15	Back		+	+	
F	Actinic keratosis;	16	Temple		+		+
	Bowen's disease	17	Nose		+		
G	Actinic keratosis;	18	Leg		+		
	Bowen's disease	19	Ear		+		
H	Actinic keratosis	20	Leg		+		
I	Squamous cell carcinoma	21	Lip	+	+	+	
J	Squamous cell carcinoma	22	Neck	+	+	+/−[c]	+/−
K	Squamous cell carcinoma	23	Hand	+	+		
Cardiac transplant recipients							
L	Actinic keratosis	24	Hand	+	+	+	
M	Bowen's disease	25	Arm		+	+	
		26	Face		+		
N	Squamous cell carcinomas	27	Neck		+		+
		28	Sternum	+	+		
		29	Temple		+		+
		30	Ear		+	+	+

[a] PCR amplification of HPV types 5, 6/11, 16, and 18.
[b] +, Positive HPV type DNA.
[c] +/−, Weak positivity; the other HPV types tested were negative.

with HPV type 5, 6/11, 16, and 18 probes, an HPV DNA was more frequently detected by PCR than by *in situ* hybridization, 25 of 29 (86%) and eight of 29 (27%), respectively. HPV 6/11 were also more common with PCR than with *in situ* hybridization (Table IV), 20 of 25 (80%) and 3 of 8 (37%), respectively. By PCR, HPV 6/11 were identified in HPV-positive samples (three of six warts, six of six actinic keratoses, four of five Bowen's disease, and seven of nine squamous cell carcinomas), whereas by *in situ* hybridization they were detectable only in two of three warts and one of four squamous cell carcinomas, the other samples being negative.

To avoid false-positive reactions of PCR, the results were examined according to the patients, the location of the lesions, and the time of excision. HPV 6/11 were found in cutaneous lesions from 14 of 22 (64%) patients with a renal transplant and three of five (60%) patients with a cardiac transplant. These biopsies were taken from various body sites. Furthermore, when several specimens of

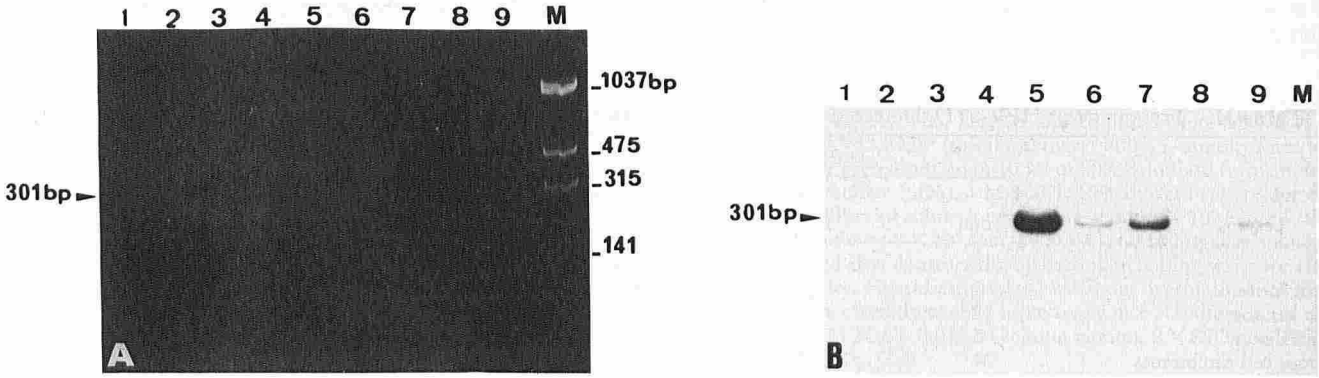

Figure 1. PCR amplification of squamous cell carcinoma DNAs from renal transplant recipients with HPV 6/11 primers. Equal amounts of amplified products were loaded and separated electrophoretically. *A*) No detection of amplified product on ethidium bromide-stained gel. *B*) Detection of amplified product on Southern blotting hybridized with ^{32}P-labeled specific oligonucleotide probe. *Lane 1,* control amplification without DNA. *Lanes 2–9,* amplified products of squamous cell carcinoma DNAs; positive reactions are seen in *lanes 5–7* (patient B) and (patient I). *Lane M,* Molecular weight markers, pBR 322 digested with Taq I restriction enzyme.

Table IV. Detection of HPV Types in Skin Lesions from Transplant Recipient by *In Situ* Hybridization and PCR

Lesions	Number	HPV Types		Total HPV DNA+[a]			HPV 6/11+[b]	
		ISH[c]	PCR[d]	ISH[c]	ISH[e]	PCR[d]	ISH[c]	PCR[d]
Warts	1	1, 6/11, 16	6/11	3/6	3/6	5/6	2/6	2/6
	2	1, 2, 5, 16	5					
	3	2, 6/11	6/11					
	4	—[f]	16					
	5	—	6/11					
	6	—	—					
Actinic keratoses	7	1,2	6/11					
	8	2	6/11	2/7	0/7	6/7	0/6	6/7
	9	—	6/11					
	10	—	—					
	11	—	5, 6/11					
	12	—	6/11					
	13	—	6/11					
Bowen's disease	14	1,2	6/11	3/6	2.6	5/6	0/6	4/6
	15	1,2,16,18	—					
	16	16	6/11, 16					
	17	—	6/11					
	18	—	16, 18					
	19	—	6/11					
SCC[g]	20	5,6/11	6/11, 18	4/10	3/10	9/10	1/6	7/10
	21	1, 16	6/11					
	22	1	18					
	23	1,2,18	6/11, 16					
	24	—	5, 6/11					
	25	—	—					
	26	—	5, 6/11					
	27	—	5					
	28	—	5, 6/11, 16, 18					
	29	—	5, 6/11					

[a] HPV DNA+, Number of lesions containing HPV DNA.
[b] HPV 6/11+, Number of lesions containing HPV types 6/11 DNA.
[c] ISH, *in situ* hybridization with detection of benign HPV types 1, 2, 6/11 and potentially oncogenic HPV types 5, 16, 18.
[d] PCR amplification of HPV types 5, 6/11, 16, and 18.
[e] ISH, *in situ* hybridization with detection of benign HPV types 6/11 and potentially oncogenic HPV types 5, 16, 18.
[f] —, no HPV DNA was detected.
[g] SCC, squamous cell carcinoma.

one patient (patient B, Table III) were studied, they were excised over a period of 3 years, and the DNA was extracted at different periods.

Three biopsy specimens (one wart, two squamous cell carcinomas) have been tested with PCR, *in situ* hybridization, and Southern blotting (Table V). By Southern blotting, only one squamous cell carcinoma (from patient 1, Table III, corresponding to case 5, Table V) was found to contain HPV types 6/11 (Fig 3), as was also shown by PCR. The wart sample was HPV 6/11 positive and was negative with other HPV types tested by PCR; the two squamous cell carcinomas contained not only HPV 6/11 DNA but also HPV 5 and/or 16 DNA. By *in situ* hybridization, HPV 6/11 was detected in only one squamous cell carcinoma and was associated with HPV 16/18; HPV types 1 and 2 were found in one sample.

Non-Transplant-Recipient Lesions A few similar lesions from controls were obtained and compared to those of transplant recipients for their content in HPV DNA type 6/11. Of the ten skin specimens tested by PCR (Table II), four (40%) contained HPV DNA; HPV 6/11 were identified only in two cases of Bowen's disease, and these lesions also contained HPV16 or 16 and 18 DNA. The eight other samples were negative with each probe tested.

Four samples (three warts and one squamous cell carcinoma) were tested by PCR, *in situ* hybridization, and Southern blotting for HPV 5, 6/11, 16, and 18 (Table V). They were also tested by *in situ* hybridization and Southern blotting with plasmid probes types 1 and 2. By PCR, all the specimens were negative. HPV type 1 and/or 2 DNA was found in warts by both *in situ* hybridization and Southern blotting.

In controls tested by PCR, *in situ* hybridization, or Southern blotting, each genital condyloma and juvenile laryngeal papilloma was found to contain HPV 6/11 DNA; they were negative for the other HPV types tested (Table II).

DISCUSSION

In this investigation we have shown that HPV types 6/11 can be detected with PCR, *in situ* hybridization, or Southern blotting in some cutaneous benign, premalignant, or malignant lesions from organ transplant recipients. The lesions appeared several years after grafting. Biopsies were taken at different body sites on sun-exposed areas. HPV type 6/11 DNAs were rarely detected in similar skin lesions from normal population.

The identification of these HPV types in cutaneous samples was unexpected because they are known to infect mucosa and are usually associated with benign lesions. On the contrary, potentially oncogenic HPV types, mainly found in mucosa, have already been found in cutaneous lesions; HPV 16, usually associated with cervical cancers [15], was detected in cutaneous Bowen's disease and squamous cell carcinomas from immunocompetent patients [8,9,16–20,21] and from renal transplant recipients [13]. HPV 16 has also been found in a benign wart from an immunosuppressed patient [7]. It is difficult to compare the frequency of HPV 16 in cutaneous samples reported by various investigators, as techniques of different sensitivity have been used, *in situ* hybridization, Southern blotting, and PCR.

The presence of HPV 6/11 in skin lesions has rarely been reported. HPV 11 DNA was mentioned in one case of verrucous carcinoma of the leg from a non-immunocompromised patient [22],

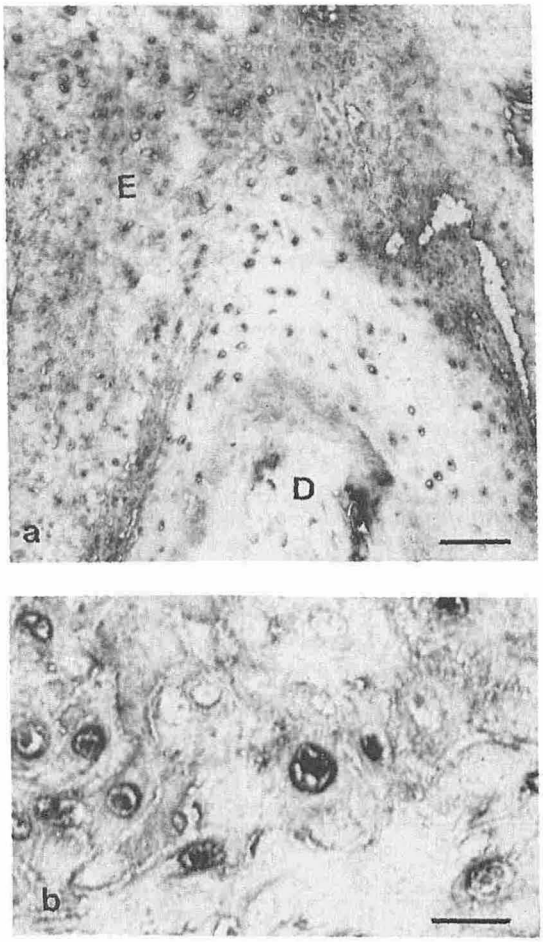

Figure 2. *In situ* hybridization with biotinylated HPV 6/11 probes under stringent conditions in a paraffin-embedded squamous cell carcinoma from a renal transplant recipient. *a*) Alkaline phosphatase staining pattern of nuclei containing HPV 6/11 DNA in epithelial cells from upper layers. *b*) Detail of positive nuclei at higher magnification. D, Dermis; E, Epidermis. *Bar*, 100 μm in (*a*) and 25 μm in (*b*).

and an HPV 6 was also found and associated with HPV 18 in one case of verrucous papules scattered over the arms and legs of a non-immunocompromised patient [23]. Both HPV 6/11 and 16 were reported in periungual squamous carcinoma *in situ* [21]. In biopsies of our study in which only HPV type 6/11 DNAs were detected, it cannot be excluded that other HPV types may be present, as only oncogenic types 5, 16, and 18 were tested from among 66 distinct HPV types so far identified [10]. In our cases, HPV 6/11 as well as potentially oncogenic HPV types 5, 16, and 18 were identified, especially in premalignant and malignant lesions. These data suggest that carriage of multiple HPVs is not uncommon in transplant recipients, although this has been described only rarely [4]. The significance of the presence of mucosal HPV 6/11 and their association with potentially oncogenic types in skin lesions remains unknown. Our findings do not establish a cooperative role or complementation of HPV 6/11 in lesions coinfected with HPV 5, 16, or 18. However, it cannot be excluded that malignancy could result from coinfection of HPV 6/11 with other oncogenic HPV types that have not been tested. The physical state of the DNA might play an important role in the evolution of these lesions towards malignancy although HPV 11 DNA has been found either integrated in cellular DNA in a perianal squamous cell carcinoma from a transplant recipient [12] or as an episomal component in a penile carcinoma [24].

In our study, among lesions from non-immunosuppressed patients, two cases of Bowen's disease were found to contain HPV 6/11 DNA; one was located on the nose, the other was on the penis.

Table V. Detection of HPV Types in Skin Lesions from Transplant Recipients by PCR, *In Situ* Hybridization, and Southern Blot

			HPV Types		
Lesions		Number	PCR[a]	ISH[b]	Southern[c] Blotting
Warts	TF[d]	1	6/11	2,5	6/11
	NTR[e]	2	—[f]	1	1
	NTH	3	—	1,2	1,2
	NTR	4	—	2	2
Squamous cell carcinomas	TR	5	5, 6/11	6/11, 16, 18	6/11
	TR	6	5, 6/11, 16	1, 2, 16, 18	—
	NTR	7	—	—	—
	NTR	8	16	—	—

[a] PCR amplification of HPV types 5, 6/11, 16, and 18.
[b] *In situ* hybridization was done with HPV type 1,2,5, 6/11, 16, and 18 probes.
[c] Southern blotting was done with HPV type 1,2,5, 6/11, 16, and 18 probes
[d] TR, transplant recipients.
[e] NTR, non-transplant recipients.
[f] —, no HPV DNA was detected with the HPV probes tested.

These two locations may explain the presence of such HPV types, which are usually identified at these sites [25,26]. The comparison with frequency of HPV 6/11 in the normal population is difficult, as these patients have single lesions instead of multiple lesions as in transplant recipients.

The common HPV 6/11 infection in skin epithelium in the transplant-recipient population could be explained by immunosuppressive treatment, which modifies the microenvironment of epithelial cells, targets of all HPV types. It is also possible that cytokines may function as tumor promoters when the immune system is suppressed [12].

The association of HPV 6/11 with cutaneous lesions from transplant recipients is clearly documented by our data, as three different techniques demonstrate HPV 6/11 DNA in some skin biopsies. The specificity and sensitivity of the PCR reaction was proved for each primer pair, using dilutions of plasmid-containing HPV DNA, as previously reported for HPV 6/11, 16, and 18 [13]. To avoid misinterpretation of results, positive (plasmid-containing HPV DNA) and negative (amplification without DNA or normal human DNA) controls were included in each amplification. To exclude contamination, DNA extraction and PCR amplification were carried out in different laboratories. Furthermore, several lesions had been excised from patient B over a period of 3 years and the DNAs were extracted at different times, thus excluding the possibility of contamination during extraction of each sample. *In situ* hybridization specificity has been proved by different controls, i.e., lesions unrelated to HPV infections such as molluscum contagiosum as well as hybridization of sections without probe or with other HPV types such as 1, 2, 5, 16, and 18 [27]. In some cases HPV DNA was detected by *in situ* hybridization but not by PCR; this could be explained by the high dilution of HPV DNA in cellular DNA or by the absence of infected cells in the part of the lesion for DNA extraction. By Southern blotting one squamous cell carcinoma from a renal transplant patient showed a typical profile of HPV 6/11; the low frequency of lesions containing HPV DNA could be explained by inadequate sampling due to the number of DNA copies diluted in cellular DNA. In previous results obtained by Southern blotting, on benign lesions from the normal population, it was shown that 48/58 skin warts (soles and hands) contained HPV DNA; they exhibited HPV type 1 in nine cases and type 2 in 34 cases, five contained both HPV 1 and 2, and none of them displayed HPV types 6/11 (unpublished results). On the contrary, non-transplant controls of condylomas and juvenile laryngeal papillomas (Table II) contained HPV 6/11 DNA but none of the other HPV DNA type tested did.

The presence of mucosal HPV 6/11 in cutaneous lesions from transplant recipients probably cannot be explained by contamination from genital site, because these patients did not have any genital

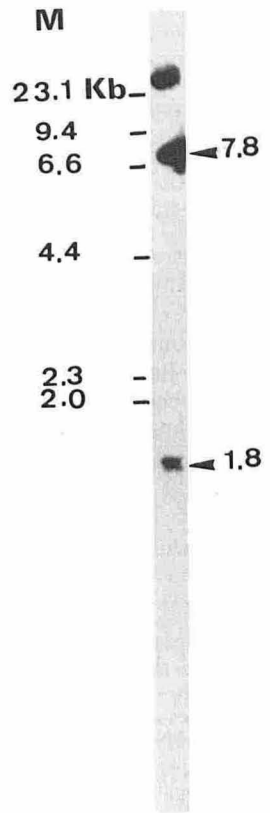

Figure 3. Example of detection by Southern blotting, under stringent conditions, of HPV 6/11 DNA, in a squamous cell carcinoma from a renal transplant recipient (I). Digestion was performed with Pst I. M, Molecular weight markers from λ phage digested with Hind III restriction enzyme.

condylomas, oral papillomas, or laryngeal papillomas, which are usually infected with these HPV types, clinically visible. As a small number of HPV types has been tested on our biopsies, it cannot be excluded that another type or a new type could be involved.

In conclusion, we report the presence of benign mucosal HPV 6/11 in benign, premalignant, and malignant skin lesions from transplant recipients; this suggests that HPVs may lose their site specificity in such patients. The significance of the presence of HPV 6/11 in cutaneous lesions remains unclear. HPV infection in itself is probably not sufficient for a progression towards malignancy; other factors such as immunosuppressive treatment, UV, or activated oncogenes probably change the epithelial cell environment.

We thank the clinicians who collected the biopsies, Dr. C. Hermier for histologic classification of the samples, and Dr. J. Carew for reviewing the English version of this manuscript.

This work was supported by INSERM, Ligue Nationale Française contre le Cancer Comité Départemental du Rhône 1991, MGEN contract 1991, and Bio-Mérieux for the primers.

REFERENCES

1. Boyle J, MacKie RM, Briggs JD, Junor BJR, Aitchison TC: Cancer, warts, and sunshine in renal transplant patients. A case-control study. *Lancet* I:702–705, 1984
2. Euvrard S: Cutaneous complications in renal transplant recipients. *Eur J Dermatol* 1:175–184, 1991
3. Rüdlinger R, Smith IW, Bunney MH, Hunter JAA: Human papillomavirus infections in a group of renal transplant recipients. *Br J Dermatol* 115:681–692, 1986
4. Van der Leest RJ, Zachow KR, Ostrow RS, Bender M, Pass F, Faras AJ: Human papillomavirus heterogeneity in 36 renal transplant recipients. *Arch Dermatol* 123:354–357, 1987
5. Barr BB, Benton EC, McLaren K, Bunney MH, Smith LW, Blessing K, Hunter JAA: Human papillomavirus infection and skin cancer in renal allograft recipients. *Lancet* I:124–129, 1989
6. Euvrard S, Chardonnet Y, Hermier C, Viac J, Thivolet J: Verrues et carcinomes épidermoïdes après transplantation rénale. *Ann Dermatol Venereol* 116:201–211, 1989
7. Gassenmaier A, Fuchs P, Schell H, Pfister H: Papillomavirus DNA in warts of immunosuppressed renal allograft recipients. *Arch Dermatol Res* 278:219–223, 1986
8. Moy RL, Eliezri YD, Nuovo GJ, Zitelli JA, Benett RG, Silverstein S: Human papillomavirus type 16 DNA in periungual squamous cell carcinoma. *JAMA* 261:2669–2673, 1989
9. Guitart J, Bergfeld WF, Tuthill RJ, Tubbs RR, Zienowicz R, Fleegler E: Squamous cell carcinoma of the nail bed: a clinicopathological study of 12 cases. *Br J Dermatol* 123:215–222, 1990
10. De Villiers EM: Heterogeneity of human papillomavirus group. *J Virol* 63:4898–4903, 1989
11. Steinberg BM: Papillomavirus. Effects upon mother and child. *Ann New York Acad Sci* 549:118–128, 1988
12. Manias DA, Ostrow RS, McGlennen RC, Estensen RD, Faras AJ: Characterization of integrated human papillomavirus type 11 DNA in primary and metastatic tumors from a renal transplant recipient. *Cancer Res* 49:2514–2519, 1989
13. Soler C, Allibert P, Chardonnet Y, Cros P, Mandrand B, Thivolet J: Detection of human papillomavirus types 6, 11, 16 and 18 in mucosal and cutaneous lesions by the multiplex polymerase chain reaction. *J Virol Methods* 35:143–157, 1991
14. Guérin-Reverchon I, Chardonnet Y, Chignol MC, Thivolet J: Study of stringency conditions for human papillomavirus DNA detection on cell lines, frozen and paraffin-embedded tissue sections by in situ hybridization with biotinylated probes. *Histochemistry* 93:637–643, 1990
15. Durst M, Gissmann L, Ikenberg H, zur Hausen H: A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci USA* 80:3812–3815, 1983
16. Stone MS, Noonan CA, Tschen J, Bruce S: Bowen's disease of the feet. Presence of human papillomavirus 16 DNA in tumor tissue. *Arch Dermatol* 123:1517–1520, 1987
17. Hahn A, Löning T, Hoos A, Henke P: Immunohistochemistry (S100, KL1) and human papillomavirus DNA hybridization on morbus Bowen and bowenoid papulosis. *Virchows Arch Pathol Anat* 413:113–122, 1988
18. Ostrow RS, Shaver MK, Turnquist S, Viksnins A, Bender M, Vance C, Kaye V, Faras AJ: Human papillomavirus-16 DNA in a cutaneous invasive cancer. *Arch Dermatol* 125:666–669, 1989
19. Guérin-Reverchon I, Chardonnet Y, Viac J, Chouvet B, Chignol MC, Thivolet J: Human papillomavirus infection and filaggrin expression in paraffin-embedded biopsy specimens of extragenital Bowen's disease and genital bowenoid papulosis. J Cancer Res Clin Oncol 116:295–300, 1990
20. Pierceall WE, Goldberg LH, Ananthaswamy HN: Presence of human papilloma virus type 16 DNA sequences in human non melanoma skin cancers. *J Invest Dermatol* 97:880–884, 1991
21. De Dobbeleer G, André J, Laporte M, Schroeder F, Peny MO, Scarcériaux B, Noël JC: Human papillomavirus type 6/11 and 16 in periungual squamous carcinoma in situ. *Eur J Dermatol* 3:12–14, 1993
22. Garven TC, Thelmo WL, Victor J, Pertschuk L: Verrucous carcinoma of the leg positive for human papillomavirus DNA 11 and 18: a case report. *Hum Pathol* 22:1170–1173, 1991
23. Blauvelt A, Duarte AM, Pruksachatkunakorn C, Leonardi CL, Schachner LA: Human papillomavirus type 6 infection involving cutaneous nongenital sites. *J Am Acad Dermatol* 27:876–879, 1992
24. Villa L, Lopes A: Human papillomavirus sequences in penile carcinomas in Brazil. *Int J Cancer* 37:853–855, 1986
25. Pater A, Gardner H, Respler DS, Jahn A, Pater MM: Isolation and characterization of a variant of human papillomavirus type 11 from a nasal inverting (Schneiderian) papilloma. *J Med Virol* 25:149–156, 1988
26. Wickenden C, Hanna N, Taylor-Robinson D, Harris JRW, Bellamy C, Carroll P, Malcolm ADB, Coleman DV: Sexual transmission of human papillomaviruses in heterosexual and male homosexual couples, studied by DNA hybridization. *Genitourin Med* 64:34–38, 1988
27. Soler C, Chardonnet Y, Guérin-Reverchon I, Chignol MC, Viac J, Euvrard S, Thivolet J: Influence of fixation on human papillomavirus DNA detection in frozen and embedded paraffin lesions by in situ hybridization on tissue sections. *Pathol Res Pract* 188:1018–1027, 1992