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In Vivo Transformation of Human Skin with Human Papillomavirus Type 11 from Condylomata Acuminata

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Human papillomaviruses (HPVs) have been implicated in the development of a number of human malignancies, but direct tests of their involvement have not been possible. We describe a system in which human skin from various sites was infected with HPV type 11 (HPV-11) extracted from vulvar condylomata and was grafted beneath the renal capsule of athymic mice. Most of the skin grafts so treated underwent morphological transformation, resulting in the development of condylomata identical to those which occur spontaneously in patients. Foreskins responded with the most vigorous proliferative response to HPV-11. The lesions produced the characteristic intranuclear group-specific antigen of papillomaviruses. Both dot blot and Southern blot analysis of DNA from the lesions revealed the presence of HPV-11 DNA in the transformed grafts. These results demonstrate the first laboratory system for the study of the interaction of human skin with an HPV. The method may be useful in understanding the mechanisms of HPV transformation and replication and is free of the ethical restraints which have impeded study. This system will allow the direct study of factors which permit neoplastic progression of HPV-induced cutaneous lesions in human tissues.

Papillomaviruses have been associated with benign, proliferative lesions of skin and other epithelia in a variety of animal species and humans (18). Over 30 types of human papillomaviruses (HPVs) are recognized, many of which are associated with distinctive lesions (20). HPVs may not be the relatively innocuous agents they were once thought to be. Recent studies have implicated HPVs in the development of premalignant and malignant lesions of the skin (7, 16), uterine cervix (4), and larynx (5).

Studies on the contribution of HPVs to the etiology of human tumors have been severely restricted by the unavailability of laboratory animal hosts or culture systems which would allow neoplastic transformation or viral replication or both. HPV infection of human subjects is ethically unacceptable. We and others have described unsuccessful attempts to develop experimental systems in which HPV-infected human tissues were grafted to immunologically deficient animals (2, 17) or privileged sites (11). Human cell cultures do not usually support growth of or transformation by HPVs (1), although limited growth of HPV in cultures of human keratinocytes has been described (14). Mouse cell transformation with HPV cloned DNA has been reported (21). Still, advances in the study of mechanisms of transformation by HPVs have been hindered by the lack of an experimental system which would allow HPV-induced changes in human tissues.

We have described the neoplastic transformation by Shope papillomavirus of normal rabbit skin grafted to athymic mice (10). That study suggested that an analogous system with human tissues and HPV might be useful. We tested that idea and found that human uterine cervical tissue can be transformed by HPV in a system using transplantation beneath the renal capsule of athymic mice (12). The HPV was extracted from a pool of vulvar condylomata acuminata and included both HPV type 11 (HPV-11) and HPV-6 DNA, demonstrated by dot blot analysis.

The purpose of the current experiments was to test the hypothesis that the same extract of human condylomata acuminata could transform normal human skin to the morphology that has been associated with HPV infections (19) and to compare the susceptibility of skin from various anatomical sites. The term transformation is used here in the literal sense to indicate a change in the morphological appearance of the infected tissues. We also identified which HPV genomes were present in the condylomata extract and compared them with the HPV genomes present in the experimentally infected human skin grafts.

MATERIALS AND METHODS

Animals. Athymic mice (*nu/nu* on BALB/c background) were purchased from Harlan Sprague Dawley, Inc., Madison, Wis. They were housed in flexible film isolators and supplied with sterile air, water, and autoclaved laboratory chow supplemented with vitamins.

Preparation of human skin grafts. Skin samples were obtained from six patients from various anatomical sites. Skin from all sites was subjected to both gross and microscopic examination and was free of detectable lesions. Split-thickness skin grafts were cut with a sterile double-edged razor blade clamped in a curved Kelly hemostat. The grafts were held in minimum essential medium with only gentamicin (800 μg/ml) as a supplement, and they were trimmed to 2 by 2 by 0.5 mm. The grafts were then rinsed and incubated for 1 h at 37°C in 1 ml of one of two treatment solutions: phosphate-buffered saline (PBS) or condylomata acuminata extract (CA). In one instance, additional grafts received herpes simplex virus type 2 (HSV-2) or both HSV-2 and CA.

Preparation of CA. Vulvar condylomata were obtained from 15 female patients and stored at −70°C until used. A total of 20 g was thawed, minced with scissors, and disrupted

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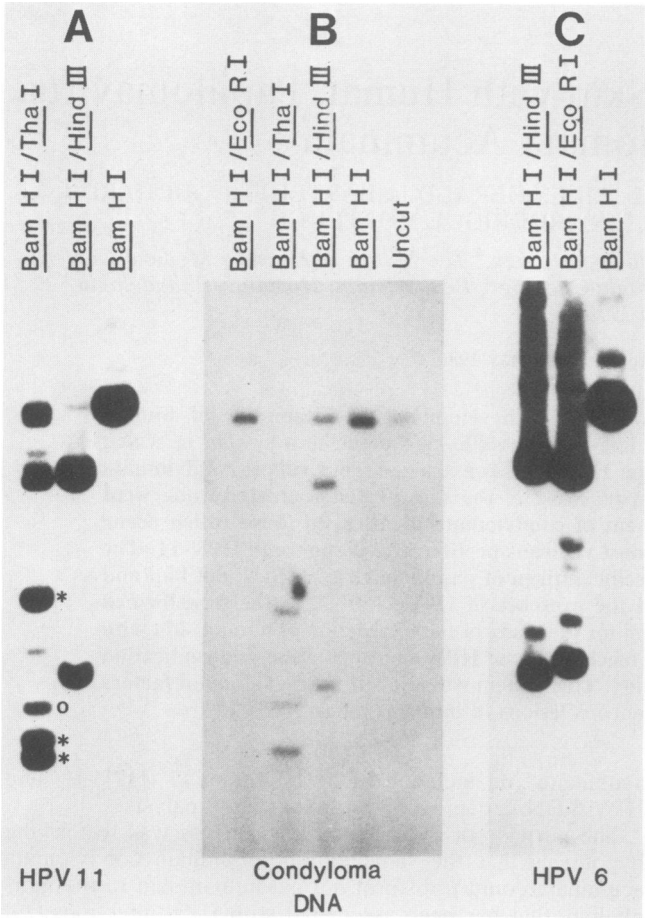

FIG. 1. Southern blot analysis of HPV DNA of infecting CA from naturally occurring human vulvar condylomata. Total DNA was extracted from the tissue pellet remaining after preparation of the extract. Of the total DNA 50% was used for the restriction endonuclease cleavage shown in panel B. Panels A and C represent control hybridizations of HPV-11 and HPV-6 to themselves. After cleavage, samples were electrophoresed on 0.8% agarose gels, blotted to nitrocellulose, and hybridized. (A) Hybridization with 5×10^5 cpm of ^{32}P nick-translated HPV-11 probe; (B) hybridization with a mixture of 5×10^5 cpm of ^{32}P nick-translated HPV-11 probe and 5×10^5 cpm of ^{32}P nick-translated HPV-6 probe; (C) hybridization with 5×10^5 cpm of ^{32}P nick-translated HPV-6 probe. The probes were purified HPV DNA inserts that were cleaved from plasmids before nick translation. Asterisks indicate three of the four products of HPV-11 cleavage by *Tha*I. The remaining (smallest) band was seen on the original blot but is not shown here. The other bands in the same lane are products of incomplete digestion. The band marked by the open circle represents incomplete digestion of the internal *Tha*I site at the junction of the smallest band and another fragment.

in 50 ml of PBS at 4°C with a Virtis homogenizer at 25,000 rpm for 30 min. Cell-free supernatants and pellets were obtained by sedimentation at $1,000 \times g$, separated, and stored at −70°C. The supernatant was used without further dilution to infect the skin grafts.

Preparation of HSV-2. HSV-2 was propagated in human diploid cells. Cell-free supernatants were obtained after the infected cells were frozen and thawed and then centrifuged. The supernatants were stored at −70°C. Plaque assays were performed on primary rabbit kidney cells, as previously described (3). Thawed HSV-2 stocks were diluted to a concentration of 1×10^7 PFU/ml and irradiated with UV light for 10 min with 46 ergs/mm^2 per s. Some skin grafts were exposed sequentially to CA followed by inactivated HSV-2; each exposure was at 37°C for 1 h.

Grafting technique. Each mouse was anesthetized with pentobarbital sodium, and the kidneys were delivered, one at a time, through dorsal, bilateral, paravertebral, subcostal incisions (12). The renal capsule was nicked, and a skin graft was placed in each kidney with toothless forceps. The skin incisions were closed with wound clips, and the mice were given drinking water with trimethoprim (0.01 mg/ml) and sulfamethoxazole (0.05 mg/ml) for the duration of the experiment.

Histological assessment of grafts. Mice were killed by cervical dislocation, and the kidneys were fixed in neutral-buffered Formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. Cohort sections were deparaffinized and incubated with antibody raised against disrupted bovine papillomavirus (Dakopatts; Accurate Chemical & Scientific Corp., Westbury, N.Y.) for demonstration by the immunoperoxidase technique of the group-specific antigen (GSA) (8, 13). The GSA is an internal capsid antigen common to most papillomaviruses (8, 13). Positive controls consisted of canine papillomas or human vulvar condylomata. Negative controls were normal human skin.

Identification of HPV genomes in CA and transformed grafts. Cloned DNAs of HPV-6, -11, -16, and -18 were graciously provided by P. Howley (National Cancer Institute, Bethesda, Md.) and H. zur Hausen (Freiburg, Federal Republic of Germany). Recombinant DNA constructs were transfected into *Escherichia coli* HB101 (15). *E. coli* was grown in bulk, and plasmids were amplified with chloramphenicol. DNA was extracted with lysozyme and Triton X-100 and purified on cesium chloride-ethidium bromide equilibrium gradients. Plasmid identities were verified by cleavage with appropriate restriction endonucleases and agarose gel electrophoresis. Purified plasmids containing HPV DNAs were ^{32}P nick translated to a specific activity of about 1×10^8 cpm/μg of DNA by using a commercial kit (Amersham Corp., Arlington Heights, Ill.). The HPV content of the infecting material was examined by Southern blot analysis. Phenol and phenol-chloroform were used to extract high-molecular-weight DNA from the tissue pellet of the homogenized condylomata pool. DNA samples were precipitated with ethanol, resolubilized, bound to nitrocellulose filters, and hybridized under stringent conditions (35.6% formamide–10% dextran sulfate–0.845 M Na^+ at 46°C) with the ^{32}P nick-translated HPV DNA probes. The filters were washed, dried, and exposed to X-ray film at −70°C.

RESULTS

Identification of HPV genomes in CA. Previous analyses of the DNA contained in the infecting extract revealed hybridization to probes of HPV-11 DNA and, to a lesser extent, HPV-6 DNA. To determine whether one or both viruses were present, the DNA solubilized from the pellet of the CA used for the infection of grafts in these and past experiments (12) was analyzed by Southern blotting. The results indicated that the DNA from the condylomata pool was not cleaved by *Eco*RI but was cleaved by *Tha*I in a manner similar to the cleavage of the HPV-11 positive control (Fig. 1). The bands marked by asterisks represent three of the four internal *Tha*I cleavage products after complete digestion of HPV-11. The fourth (smallest) band was seen on the original

TABLE 1. Morphological transformation, GSA expression, and HPV DNA content of human skin grafts infected with HPV from condylomata acuminata and transplanted beneath the renal capsule of athymic mice[a]

Graft treatment	Survival frequency[b]	Graft diam (mm [mean ± SEM])	No. of grafts[c]: Trans-formed	GSA positive	In which HPV-11 genome detected[d]
PBS	2/6	4.0 ± 2	0/2	0/2	0/1
HSV-2[e]	5/6	3.6 ± 1	0/5	0/5	0/1
CA	5/5	7.6 ± 1	3/5	5/5	2/2
HSV-2 + CA	9/12	7.5 ± 2	5/9	8/9	0/2

[a] Foreskin from a 29-year-old male was used. All grafts were harvested between 96 and 128 days of growth.
[b] Number of grafts which survived per total number of grafts.
[c] Number of samples which were positive per total number tested.
[d] For these data, the two grafts from each mouse were pooled for each sample.
[e] UV irradiated.

blot but is not visible in Fig. 1. The band marked by the open circle represents incomplete digestion of the internal *Tha*I site at the junction of the smallest band and another fragment. All of the remaining bands in the lane representing the *Bam*HI/*Tha*I cleavage of HPV-11 were products of incomplete digestion. These results suggested that HPV-11 DNA, but not HPV-6 DNA, was detected in the condylomata pool used as the infecting agent in these experiments.

Transformation of adult foreskin with papillomavirus. The foreskin of a 29-year-old male was excised for phimosis. Split-thickness skin grafts were cut and exposed to PBS, HSV-2, CA, or HSV-2 and CA combined. The grafts were transplanted beneath the renal capsule of athymic mice and harvested between 96 and 128 days of growth (Table 1). Infection with the CA produced a twofold enlargement in average graft diameter and caused morphological transformation in three of five grafts; five of five grafts were positive for the GSA. Cells which were positive were abundant and found exclusively in the maturing, koilocytotic and parakeratotic layers. Both of the samples from the four paired transformed grafts that were tested hybridized with the HPV-11 and HPV-6 probes but did not react with the HPV-16 and HPV-18 probes. This pattern of dot blot reactivity was identical to that seen in the infecting inoculum. The grafts which were infected with both HSV-2 and CA also showed a twofold enlargement in average graft diameter, and five of nine grafts were morphologically transformed. Eight of nine grafts were positive for the GSA, but neither of the two grafts tested reacted with the HPV probes. Insufficient DNA sample recovery may explain the negative results for these two samples. Treatment with PBS or HSV-2 alone induced no changes in the specific endpoints.

Transformation of child foreskins with papillomavirus. Two siblings 8 and 23 months old received elective circumcisions. Split-thickness skin grafts were cut from the specimens. The grafts were exposed to CA or PBS and grafted beneath the renal capsule of athymic mice. After 98 to 130 days of growth the grafts were examined. All of the 12 grafts from the 8-month-old male survived, whether they were from the control or CA-treated groups (Table 2). The CA-infected grafts were about twofold larger in average diameter than the controls. Examples of several enlarged grafts are presented in Fig. 2. Four of the six infected grafts were both morphologically transformed and GSA positive. Microscopically, the PBS-treated grafts were keratin-filled cysts lined by a slightly hyperplastic epithelium (Fig. 3A). The CA-infected cysts were extremely hyperplastic, and the epithelium often contained papillary fronds with overlying parakeratosis (Fig. 3B). Cells of the maturing layers contained clear, perinuclear spaces with pyknotic, wrinkled nuclei (Fig. 4A) characteristic of koilocytosis (19). The immunoperoxidase test for the GSA demonstrated strong, positive staining of the nuclei in the maturing and keratinized layers; the cells of the proliferating, basal layers were negative (Fig. 4B). The three CA-infected samples hybridized with both HPV-11 and HPV-6 but did not react with HPV-16 or HPV-18 (Table 2). The control grafts were negative for all endpoints studied.

All of the grafts from the 23-month-old sibling also survived for 98 to 130 days, when they were harvested (Table 2). The CA-infected grafts were threefold larger in average diameter than the controls. Of the CA-infected grafts, 9 of 11 were morphologically transformed and 9 of the 10 examined were GSA positive. The HPV-11 and HPV-6 probes hybridized (dot blot) with all four of the CA-infected samples, but the samples did not hybridize with HPV-16 or HPV-18 (data not shown). The PBS-treated grafts morphologically resembled normal foreskin and were negative for all endpoints studied (Table 2). Three foreskin grafts from this patient were also subjected to Southern blot analysis after cleavage with restriction endonucleases. The results for two of the grafts are shown in Fig. 5 and demonstrate that the transformed graft DNA was not cleaved by *Eco*RI but was cleaved by *Tha*I in a manner similar to the cleavage of the HPV-11 positive control. The asterisks mark three of the four products of HPV-11 cleavage by *Tha*I. The fourth (smallest) band ran off the gel. The faint signal seen with the largest *Bam*HI-*Tha*I cleavage product was not expected and was probably a result of inefficient transfer of the larger fragment during blotting. This result confirmed the presence of HPV-11, but not HPV-6, in the transformed graft. Thus, only HPV-11 was detectable in the original, infecting CA, and only HPV-11 was found in the grafts which were transformed with this material.

Transformation of vulvar skin with papillomavirus. Split-thickness skin grafts were cut from a vulvectomy specimen freshly excised because of squamous cell carcinoma in an 81-year-old female. Only uninvolved skin was selected for experimental studies. Grafts were either exposed to PBS or infected with CA. After 96 to 128 days of growth beneath the renal capsule, the grafts were removed and examined (Table 2). There were no differences in the average sizes of the CA-infected or control grafts. Of 13 CA-infected grafts, 4 were both morphologically transformed and GSA positive. The CA-infected sample tested was positive when hybridized with the HPV-11 and HPV-6 probes but did not react with HPV-16 or HPV-18. All endpoints were negative in the PBS-treated control grafts.

Transformation of abdominal skin with papillomavirus. Abdominal skin from an 80-year-old female who died of myocardial infarct was obtained from the midline epigastrium during autopsy. Split-thickness skin grafts were prepared and infected with the CA as described above. The infected and control grafts were harvested after 42 to 158 days of growth beneath the renal capsule of athymic mice (Table 2). Most of the grafts survived the growth period and formed keratin-filled epidermal cysts. There were no differences in the survival frequency or sizes of the CA-infected or control grafts. None of the histological changes which are associated with condylomatous transformation were found in any of the grafts. Of the nine infected grafts, two were positive for the GSA, despite the lack of morphological

TABLE 2. Morphological transformation, GSA expression, and HPV DNA content of human skin grafts infected with HPV from condylomata acuminata and transplanted beneath the renal capsule of athymic mice

Source of graft (patient)	Treatment	Survival frequency[a]	Days harvested (range)	Graft diam (mm [mean ± SEM])	No. of grafts[b]:		
					Trans-formed	GSA positive	In which HPV-11 genome detected[c]
Foreskin (8-mo-old male)	PBS	6/6	98–130	4.3 ± 0.7	0/6	0/6	0/3
	CA	6/6	98–130	8.3 ± 1.4	4/6	4/6	3/3
Foreskin (23-mo-old male)	PBS	12/12	98–130	3.0 ± 0.3	0/11	0/7	0/3
	CA	11/11	98–130	10.6 ± 1.1	9/11	9/10	4/4
Vulva (81-yr-old female)	PBS	10/10	96–128	3.9 ± 0.3	0/10	0/4	0/1
	CA	12/13	96–128	4.2 ± 0.3	4/13	4/13	1/1
Abdominal skin (80-yr-old female)	PBS	9/10	88–158	5.6 ± 0.8	0/9	0/9	0/4
	CA	9/12	42–158	4.8 ± 0.5	0/9	2/9	3/3
Lower leg skin (80-yr-old male)	PBS	13/22	91–150	4.7 ± 0.7	0/13	0/12	0/4
	CA	20/24	91–150	4.8 ± 0.4	2/20	2/19	3/5

[a] Number of grafts which survived per total number of grafts.
[b] Number of samples which were positive per total number tested.
[c] For these data, the two grafts from each mouse were pooled for each sample.

transformation. DNA from eight control grafts (paired from the right and left kidneys of a single nude mouse) and six CA-infected grafts (similarly paired) was analyzed by dot blot for sequences hybridizing with the HPV probes. All three of the paired samples from the six CA-infected grafts hybridized with probes of HPV-11 DNA and to a lesser extent HPV-6 DNA, but they did not react with the HPV-16 or HPV-18 probes. All of the control samples failed to react with any of the HPV probes.

Transformation of lower leg skin with papillomavirus. Split-thickness skin grafts were cut from the pretibial region of a lower limb which had been freshly amputated from an 80-year-old male with peripheral vascular disease. The skin grafts were exposed to either the CA or PBS and grafted beneath the renal capsule of athymic mice. The grafts were harvested after 91 to 150 days of growth. There were no significant differences in the frequencies of graft survival or the sizes of the grafts at harvest. Of 20 grafts infected with CA, 2 were morphologically transformed as determined by the usual histological criteria (Table 2). The two grafts were also positive for the GSA. None of the control grafts was morphologically transformed or GSA positive. Dot blot analysis revealed that three of the five samples were positive for both HPV-11 and HPV-6, but they did not hybridize with the HPV-16 or HPV-18 probes. None of the control samples reacted with any of the probes.

FIG. 2. Cysts which developed from grafts of human foreskin of 23-month-old patient. The grafts were placed beneath the renal capsule of athymic mice 130 days previously. The four grafts on the left were uninfected controls, whereas those on the right were infected with the CA. The virus-infected grafts were at least twice the diameter of the uninfected grafts, and the kidneys were effaced by the expanded cysts.

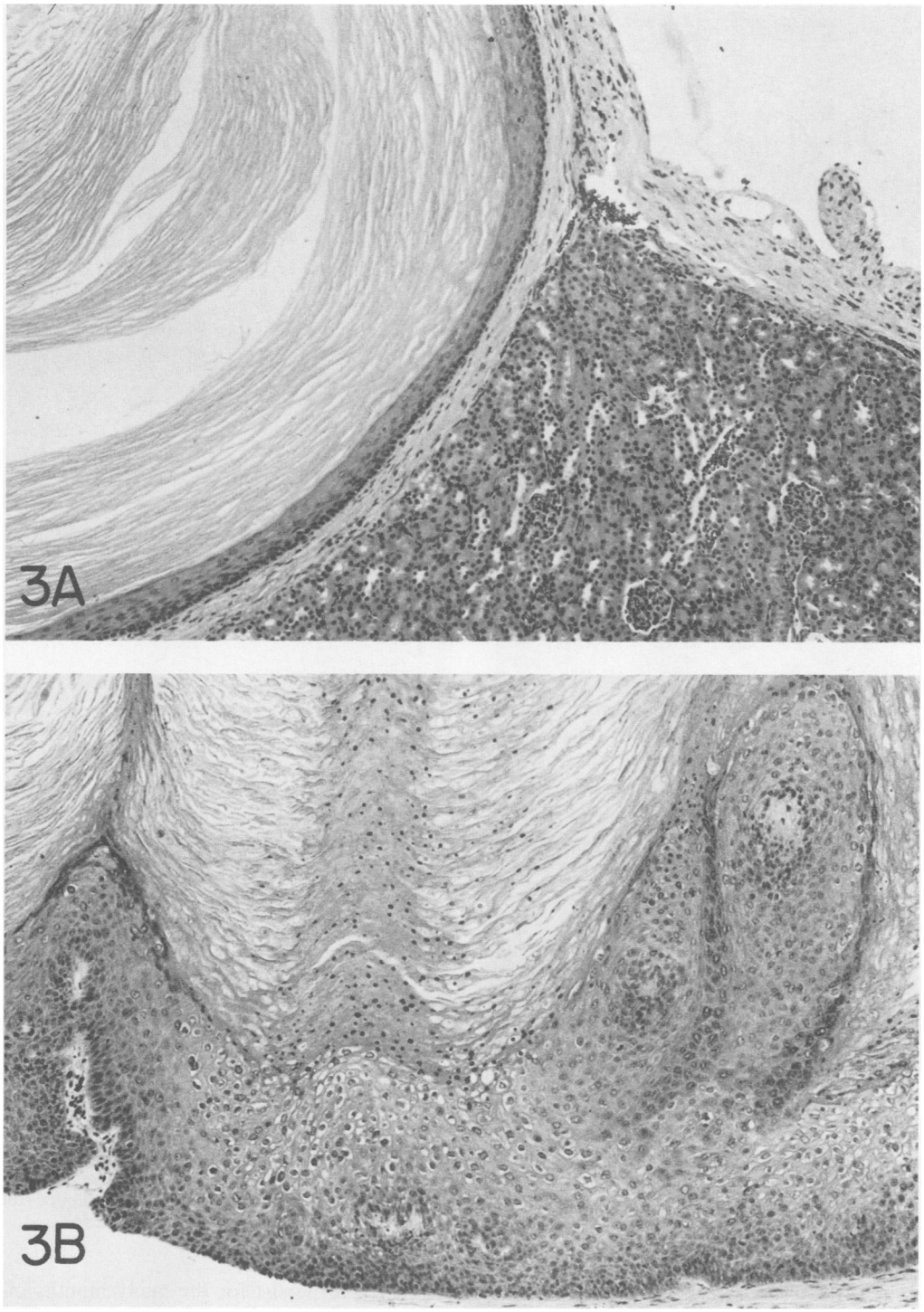

FIG. 3. (A) Section of normal epithelium which persisted 152 days after untreated human foreskin from a 29-year-old patient was grafted beneath the renal capsule of an athymic mouse. The cyst wall is composed of a normal, stratified squamous epithelium which lines a cavity filled with keratin. The kidney can be seen in the lower right corner. The renal capsule overlies both the kidney and graft (upper right). Stain, hematoxylin and eosin; magnification, ×80. (B) Section of papillomatous epithelium present 152 days after grafting CA-infected foreskin from the same patient. The epithelium is markedly hyperplastic, with papillary fronds projecting into the keratin-filled interior. Stain, hematoxylin and eosin; magnification, ×80.

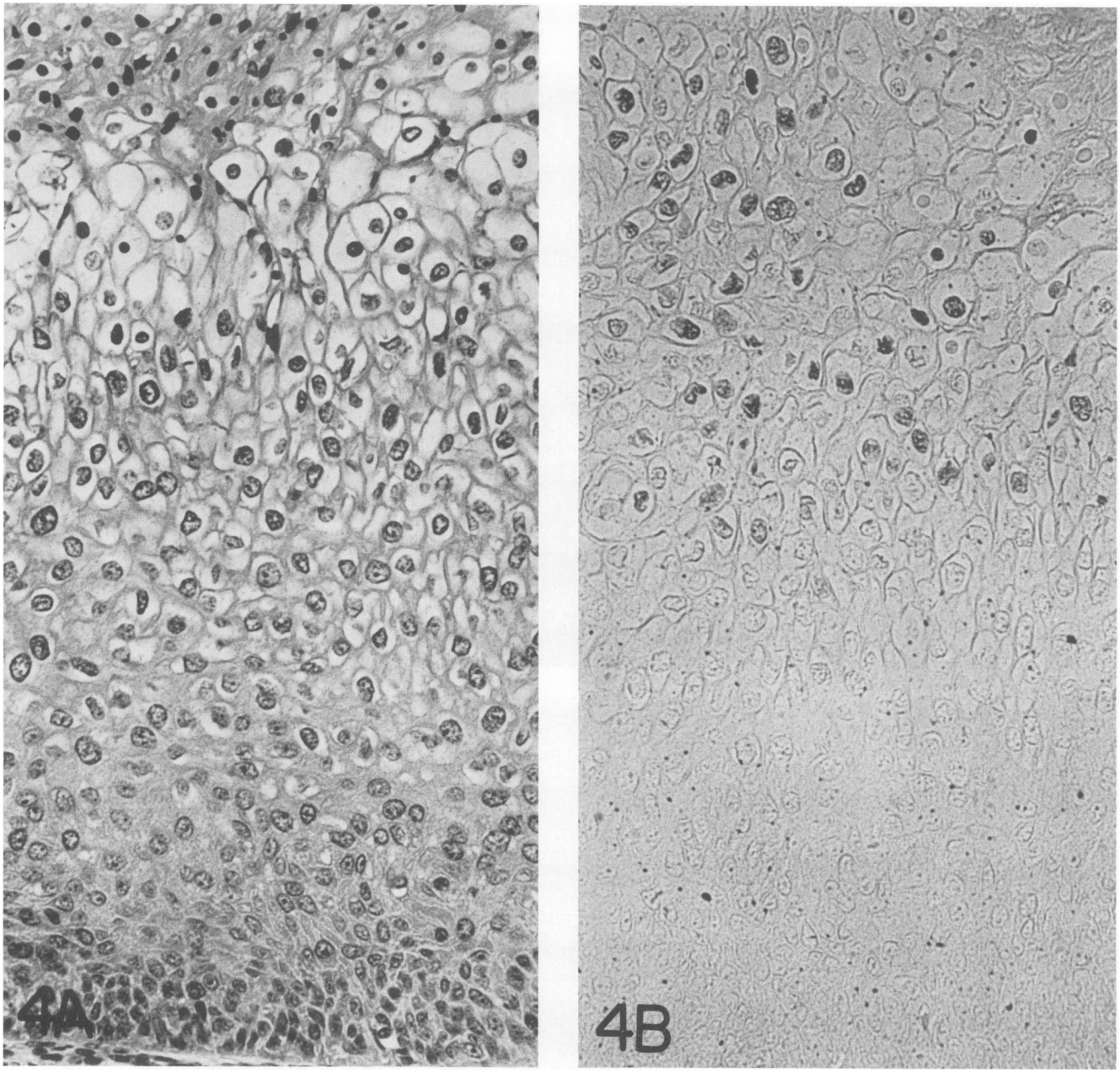

FIG. 4. (A) Papillomatous epithelium of foreskin graft from 8-month-old boy infected with CA. The proliferating, basal layers of the papilloma are hyperplastic and form a smooth transition into a broad zone of koilocytotic cells. These cells have hyperchromatic nuclei with irregular contours and clearing (vacuolization) of the perinuclear cytoplasm. Binucleation is common. There is overlying parakeratosis. The keratin layer is parakeratotic. Stain, hematoxylin and eosin; magnification, ×256. (B) Same section as in panel A reacted with antibody against GSA and subsequently treated with immunoperoxidase reagents. The dark nuclei in the upper regions of the koilocytotic layer reacted positively. Note the absence of reactivity in the proliferating lower layers. Unstained section; magnification, ×256.

DISCUSSION

This is the first demonstration in experimentally infected human skin of morphological transformation by HPV and expression of the GSA. The distribution of the GSA in infected grafts provides strong support for the inference that HPV replication occurred therein. The only alternative to this conclusion is that the GSA represented input (nonreplicating) virus from the original exposure. This interpretation is untenable because the input virus would not be expected to persist for the many months of growth of the grafts in the mice. Even if the input virus had persisted, its distribution should have been random. The distribution of viral antigen only in the differentiating layers and its absence from the lower, proliferating cells, precisely as was described for human condylomata (13), cannot be explained except by HPV replication.

In a previous study (12) we demonstrated that DNA obtained from the infecting CA hybridized with nick-translated probes of both HPV-11 and HPV-6. In the present

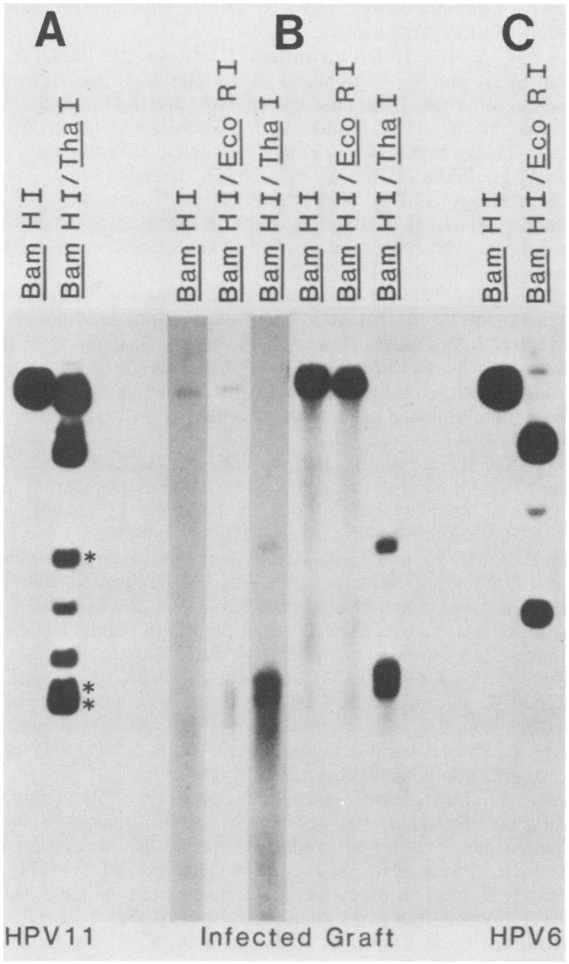

FIG. 5. Southern blot analysis of HPV DNA of transformed foreskin graft. Total DNA was extracted from the tissue. Panels A and C represent control hybridizations of HPV-11 and HPV-6 to themselves. After cleavage, samples were electrophoresed on 0.8% agarose gels, blotted to nitrocellulose, and hybridized. (A) Hybridization with 5×10^5 cpm of ^{32}P nick-translated HPV-11 probe; (B) hybridization with a mixture of 5×10^5 cpm of ^{32}P nick-translated HPV-11 probe and 5×10^5 cpm of ^{32}P nick-translated HPV-6 probe; (C) hybridization with 5×10^5 cpm of ^{32}P nick-translated HPV-6 probe. The probes were purified HPV inserts that were cleaved from plasmids before nick translation. The asterisks are defined in the legend to Fig. 1.

study, we used Southern blot analyses combined with endonuclease digestion to demonstrate that the CA contained only HPV-11. Our extract was obtained from 20 g of condylomata excised from 15 patients. Most condylomata studied by others contained little virus, and vulvar condylomata much more frequently contained HPV-6 than HPV-11 (6). Thus, the apparent absence of HPV-6 in our extract suggests that most of the infectious HPV-11 may have been contributed by one or very few condylomata. HPV-11 was also recovered from most of the transformed grafts in our studies. HPV-6 hybridizations in dot blot analyses could be attributed to cross-reactions due to the 25% overlap between the HPV-11 and HPV-6 genomes (7). This inference was strongly supported by Southern blot analysis and endonuclease digestion of the infected grafts. Transfection of cloned HPV DNA into human tissue grafts is the only way to unequivocally exclude coinfection with additional HPVs. We cannot formally exclude the possibility that HPV-11 free DNA was responsible for the infectivity of the condylomata extract, but this seems unlikely because of the abundance of nucleases in such homogenates. The HPV-11 genome has been demonstrated in vulvar and cervical condylomata, laryngeal papillomata, and cervical carcinomas (6, 18, 20). Some investigators believe that these associations imply an etiological relationship. Our experiments with HPV-11 in cervical tissue (12) and in skin in the present study provide strong support for this conclusion since our observations fulfill Koch's third postulate (9).

HSV-2 has been implicated in the development of carcinoma of the uterine cervix (3). UV light irradiation destroys HSV-2 cytolytic activity at a faster rate than HSV ability to transform hamster cells in vitro (3). We included HSV-2 treatment of some of the foreskin grafts to test the possibility that joint infection with both viruses might produce malignancy, as was recently proposed by zur Hausen (22). However, the results did not reveal any changes in the morphology of the condylomatous transformation which followed HPV-11 infection. We cannot exclude the possibility that a more prolonged period of growth might have altered the outcome.

We found that foreskins were the most sensitive human skin specimens to transformation by HPV-11. Infected tissue from 23- and 8-month-old boys and a 29-year-old male formed epithelial cysts two to three times larger than control cysts from the same patients. In contrast, the epithelial cysts of the abdominal skin, lower leg, and vulva failed to show a size differential. The foreskin specimens appeared to contain proportionally more GSA-positive nuclei than did infected skin cysts from other sites. It is not yet possible to titrate the quantity of HPV extracted from tissues, but these observations suggest that HPV-11 might replicate more abundantly in foreskins than in skin from other sites. This raises the possibility that HPV-11 might be able to discriminate between human epidermal cells from various sites. However, there were also substantial age differences among the skin donors in these experiments, and it is possible that the age differences were a more important variable than the sites. Because of ethical restrictions, it is usually impossible to obtain skin from the same donor at multiple sites.

Some of the HPV-11-infected abdominal skin cysts did not show morphological transformation but were positive for the GSA or for HPV genomes. Thus, morphological transformation is a more conservative endpoint than the production of the GSA or HPV genomes. This finding may be attributed to the fact that both assays are more sensitive than the morphological change, which requires the participation of large numbers of cells. The GSA assay can detect changes in single cells, and the dot blot assay is sufficiently sensitive in our hands to detect one HPV genomic equivalent per cell.

Our studies also indicated that the keratin-filled cysts of the transformed grafts contained abundant GSA-positive nuclei. This clearly demonstrates viral capsid antigen production and suggests that such grafts might provide an important source of experimentally produced infectious HPVs and capsid-associated antigens for future studies. We have recently extracted infectious HPV-11 from laboratory lesions, purified the virus on cesium chloride density gradients, and demonstrated virions by electron microscopy. These results will be reported elsewhere.

Foreskin offers many advantages as a substrate tissue for infection with HPV-11 and possibly other HPVs. It is much

more readily available than cervical tissue or skin from other sites. There are no preexisting diseases, especially with neonatal foreskin. Microbial contamination is usually manageable. Foreskin may be stored for several days with minimal loss of viability. Finally, foreskin responds to HPV-11 infection with much more exuberant proliferation than any other tissue which we have tested.

The experiments reported here involved a novel approach to the analysis of HPV infection of human skin. This system obviates the ethical restrictions imposed on inoculation of human subjects and permits the study of the pathogenesis of putative HPV-induced cutaneous lesions of humans. The contribution of additional factors such as other viruses, UV light, and chemical carcinogens to the malignant conversion of benign HPV-induced lesions may also be defined.

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