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Association of Human Papillomavirus Type 16 With Neoplastic Lesions of the Vulva and Other Genital Sites by In Situ *Hybridization*

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The authors examined paraffin sections from 85 genital tract tissues from 49 cases for the presence of human papillomavirus (HPV) Types 6/11, 16, and 18 by stringent *in situ* hybridization using ^{35}S-labeled viral DNA probes, and for viral capsid antigen by the immunoperoxidase test. The cases, selected mostly on the basis of vulvar pathology, were distributed as follows: early neoplasia (Group I, 6 cases); early neoplasia with viral cytopathic effect (CE) (Group II, 24 cases); and papillomavirus infection (PVI) (Group III, 19 cases). Available tissues from all affected sites were examined when the disease was multicentric. One or more viral DNAs were identified in 58% of 77 tissues from Groups II and III and in 2 of 8 tissues from Group I. HPV-6/11, HPV-16 and HPV-18 DNAs were detected, respectively, in 25, 24, and 2 tissues; 3 tissues were infected simultaneously with either two or three viruses. Viral DNA was identified at more than one site in 14 of 30 DNA-positive patients; in 10 of these, a single type was detected at all sites in the same patient. The viral DNA was localized mostly in areas showing viral cytopathology. The presence of HPV-16 correlated with neoplasia. HPV-16 DNA was identified in the 2 virus-positive tissues showing neoplasia, in 17 of 20 (85%) of the DNA-positive tissues showing neoplasia with CE, and in 5 of 25 (20%) of the DNA-positive tissues showing PVI. Conversely, HPV-6/11 was found in 25% of the DNA-positive tissues showing neoplasia with CE and in 80% of the cases of PVI. An HPV genome was identified in neoplastic cells in 14 instances; in all but 1 case, the genome was HPV-16. The association of HPV-16 with neoplasia was seen for both vulvar and cervical lesions. Viral antigen was detected in 83% of lesions associated with HPV 6/11 and in 62% of lesions associated with HPV-16. (Am J Pathol 1987, 127:206–215)

CERVICAL intraepithelial neoplasia (CIN) is widely recognized as the precursor lesion of invasive carcinoma of the cervix,[1] and human papillomavirus (HPV) Types 16 and 18 are strongly associated with both preinvasive and invasive lesions of the cervix.[2,3] The progression of vulvar intraepithelial neoplasia to invasive cancer is less clearly documented,[4,5] and there are few virologic studies of these conditions.[6–9] The technique of *in situ* hybridization of paraffin sections with HPV DNA probes[10–13] permits a retrospective analysis of routinely collected pathologic specimens for the diagnosis of infecting viral types and also allows localization of the genome to specific cells in the tissue. In an earlier investigation, Pilotti et al[8] reported that vulvar neoplasias frequently display areas of viral cytopathic effect (CE) and that virus particles and viral antigens are readily detected in a high proportion of the tissues which show these effects. In order to elucidate the relationship between viral types and vulvar neoplasia, we employed the *in situ* hybridization test to compare the distribution of HPV types in affected tissues derived from three groups of patients: those showing only neoplasia, those showing neoplasia with viral cytopathic effect (CE), and those showing only papillomavirus infection (PVI). The results indicate that HPV-16 is the virus predominantly associated with vulvar neoplasia

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and that HPV-6/11 is associated with PVI lesions. Production of capsid antigen was detected as often in HPV-16 associated lesions as in those associated with HPV-6/11.

Materials and Methods

Eighty-five tissues from 49 patients were studied. Patients were selected on the basis of the pathologic diagnosis of the vulva (42 cases) or the cervix (7 cases) according to criteria described earlier.[1,8] All available tissues were examined when the disease was multicentric. Three groups of patients were studied.

Group I: Neoplasia (6 Cases, 8 Tissues)

There were 6 cases of early vulvar carcinoma (3 of carcinoma *in situ* and 3 of early invasive carcinoma). Lesions were without areas of viral cytopathic effect. Three of the cases also had CIN Grade III.

Group II: Neoplasia With CE (24 Cases, 39 Tissues)

There were 20 cases of early vulvar carcinoma (9 of carcinoma *in situ* and 11 of early invasive carcinoma) and 4 cases of CIN Grade III. All cases showed areas of CE. Fifteen cases had multicentric disease.

Group III: Papillomavirus Infection (PVI) (19 Cases, 38 Tissues)

There were 16 cases of the vulva and 3 of the cervix. These cases showed histologic features of acuminate or flat condylomas and were without abnormal mitotic figures. Eighteen cases involved multicentric disease.

In Situ Hybridization

Adjacent sections on separate slides from each paraffin block were hybridized with ^{35}S-labeled HPV-6/11, HPV-16, HPV-18, and pBR probes under stringent conditions (Tm-17 C). The HPV-6, 11, 16, and 18 clones in pBR322 were kindly supplied by Dr. H. zur Hausen. HPV-6 and HPV-11 were used as a mixed probe because these two closely related viruses cross-hybridize to some degree even under stringent conditions.[14,15] The pBR322 DNA probe served as a negative control. We hybridized known HPV-positive sections with homologous and heterologous probes to monitor the specificity of the hybridization.

The protocol used in our laboratory is a modification of the procedure described by Brahic and Haase[16] and has been described in detail.[11] Briefly, paraffin sections were cut with the use of 0.1% Elmer's glue in the waterbath, placed on poly-D-lysine-coated slides, and heat-fixed. To prepare them for the *in situ* test, we dewaxed them, hydrated them in graded ethanols, and air-dried them. They were treated with a mild acid, then with a proteolytic enzyme, and acetylated. The HPV DNA probes were prepared by nick translation of viral DNAs cloned in pBR322 vector with one or two ^{35}S-labeled nucleotides and had specific activities of 1.5 to 5 $\times$ 10^8 cpm/μg. The probes, in a hybridization mixture, were pipetted onto the slides, which were covered with siliconized coverslips and sealed with rubber cement. The probe and tissue DNA was denatured simultaneously by floating in a 90 C waterbath. After 2 days of hybridization, the unbound probe was removed by extensive washing. Slides were dehydrated, dipped in photographic emulsion, dried, and allowed to autoradiograph for 2–5 days in light-tight boxes at 4 C. Finally, they were developed and counterstained with hematoxylin.

Immunoperoxidase Tests

Paraffin sections were examined for viral capsid antigen by the avidin–biotin complex immunoperoxidase technique as described.[17] A broadly cross-reactive, genus-specific rabbit antiserum, prepared by immunization with disrupted capsids, was used to identify the viral antigen.

Results

Characteristics of Hybridization

Specific hybridization was seen as a concentration of silver grains over nuclei of squamous cells in areas of condylomatous change or intraepithelial neoplasia. It was not seen in morphologically normal squamous epithelium, in columnar cells, or in the underlying stroma. Forty-four of the 47 DNA-positive tissue specimens were positive with only one probe; two reacted with two probes, and one reacted with three probes, indicating multiple infections at a site. In two instances of multiple infections at a site, the labeled cells were in adjacent areas from the same block or in sections of different blocks from the same site. In the third case, both probes (HPV-16 and HPV-18) labeled cells in identical areas. The pattern of labeling was focal. A heavily labeled cell was often surrounded by morphologically identical, unlabeled cells (Figure 1), and typically the label was present in only some areas of an affected tissue. Some positive specimens exhibited only a few positive cells on the surface, whereas others showed positive cells extend-

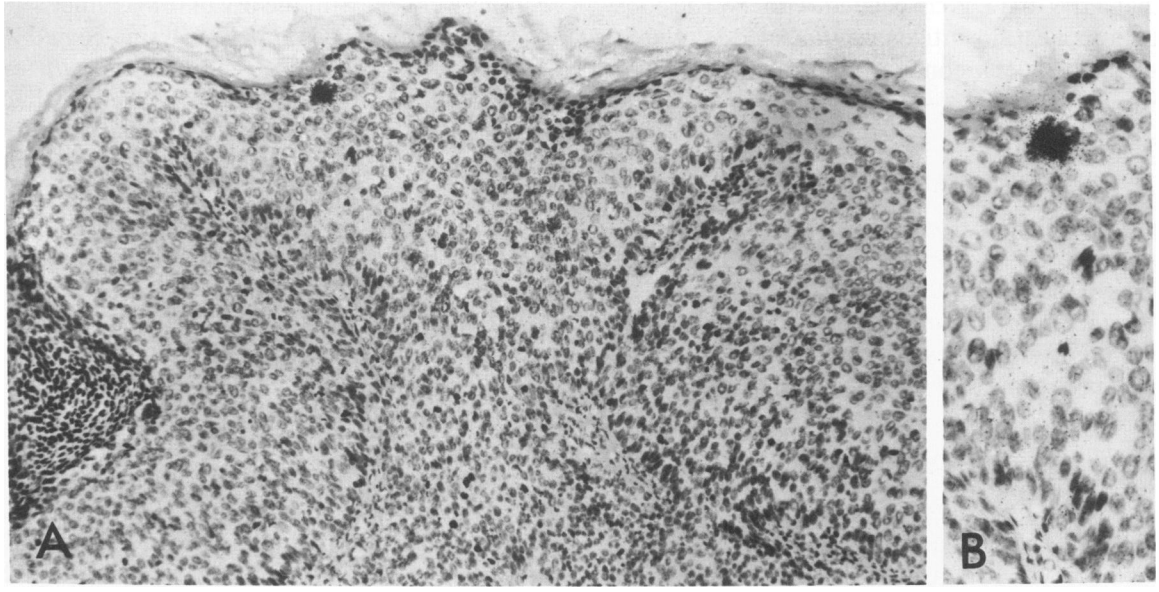

Figure 1—Area of PV-associated VIN III (Case 7) hybridized with HPV-16 probe. **A**—One strongly positive cell is located in the proximity of the thin layer of maturation. (×120) **B**—A higher power view of the positive area. (×250)

ing the full depth of the epithelium, occasionally including the basal layer (Figure 2B). The labeling was always strongest toward the surface and became progressively lighter toward the basal layer. When a tissue was positive for both DNA and antigen, the DNA was detected in many more cells and extended deeper into the epithelium than the antigen. The intensity of hybridization varied widely, from barely detectable above the background level to thousands of grains over each cell, obscuring cellular detail. Intense hybridization was usually seen in PVI associated with no or minimal atypia, especially in areas which were antigen-positive (Figures 3–6); it was also seen sometimes in antigen-negative specimens. In severe atypias, the heaviest labeling was in areas which had a lower degree of atypia and more pronounced viral CE (Figures 6 and 7). In areas of carcinoma *in situ*, there were either no positive cells or a few positive cells at the surface (Figure 1). In a few cases of carcinoma *in situ*, a variable degree of hybridization was seen over numerous cells throughout the full depth of the epithelium (Figure 2). The foci of invasive carcinoma were essentially negative. Focal, nonspecific radiolabeling was frequently seen over polymorphonuclear leukocytes and macrophages in the inflammatory infiltrate underlying carcinoma *in situ* or surrounding areas of microinvasion. These cells reacted with all HPV probes and the pBR probe.

Relationship of HPV Type to Morphology of the Lesion

In the 6 patients in Group I, viral DNA (HPV-16) was detected in 2 patients. One of the positive cases involved vulvar CIS with microinvasion; the other involved CIS only. Positive hybridization was localized to the most superficial cells in areas of VIN III. In contrast, 14 of 24 patients in Group II and 14 of 19 patients in Group III had at least one tissue that was positive for viral DNA. The pathologic features of the affected sites and the specific HPV types in these lesions for all 30 DNA-positive patients are listed in Table 1. The multicentric nature of the disease is evident for many of the cases. In most instances, the patients had similar pathologic features at the different affected sites; ie, an individual who had neoplasia with CE at one site tended to have a similar condition at other affected sites. Few patients (for example, Patients 5, 13, and 17) had PVI at one site and neoplasia with CE at another site. HPV-16 was the predominant virus in patients of Group II (Patients 3–17) and in tissues showing neoplasia with CE. Conversely, HPV-6/11 was the predominant virus in patients of Group III (Patients 18–30) and in tissues showing PVI. However, HPV-6/11 was the only virus identified in Patient 5, who had very severe multicentric disease with some sites showing neoplasia with CE

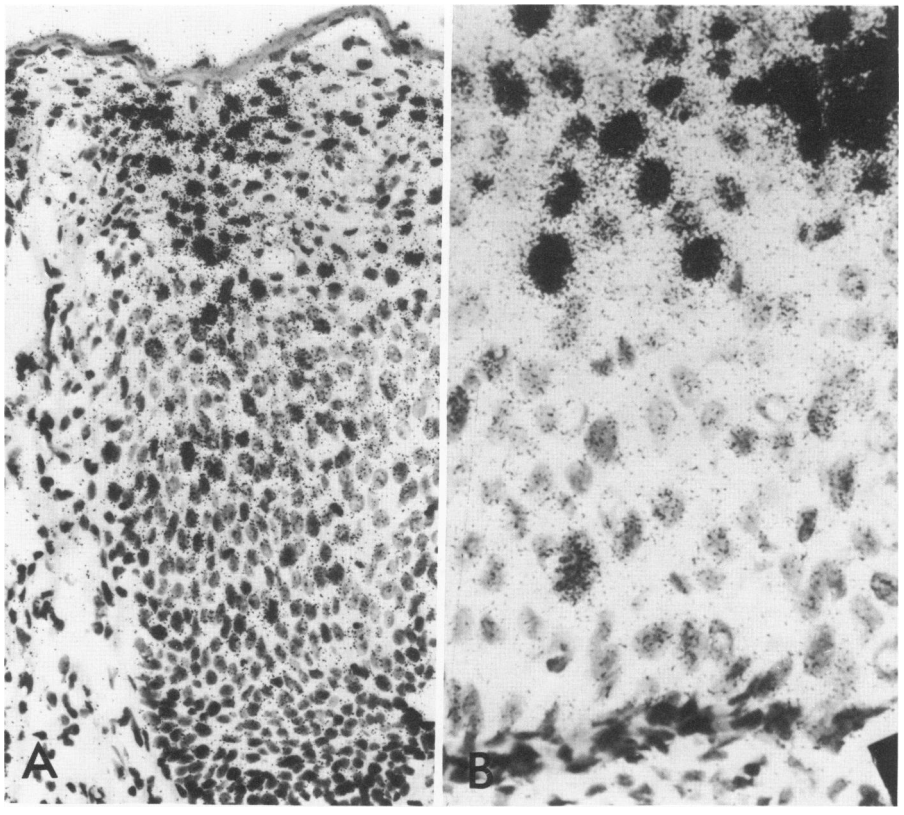

Figure 2—An area of VIN III (Case 3) hybridized with HPV-16 probe. **A**—A low-power view demonstrates positive cells the full depth of the epithelium, strongest toward the surface. (×270) **B**—A higher power reveals hybridized cells down to the basal layer. (×787)

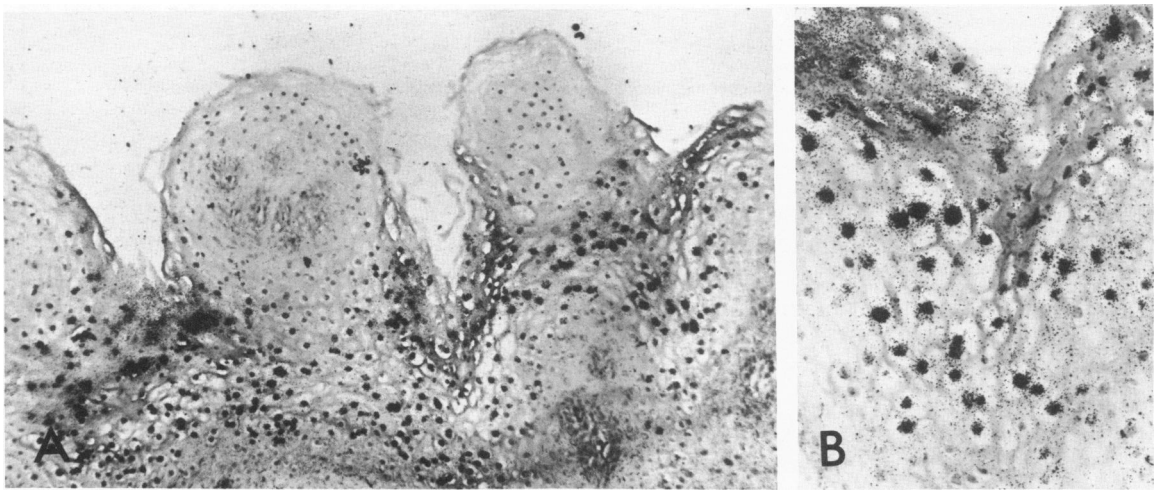

Figure 3—Buschke-Lowenstein giant condyloma of the vulva (Case 5). **A**—A low-power view of a condylomatous area showing numerous nuclei hybridized with HPV-6/11 probe, mostly in the parakeratotic layers. Hematoxylin counterstain. (×100) **B**—A higher magnification showing positive koilocytes and dyskeratocytes. (×200)

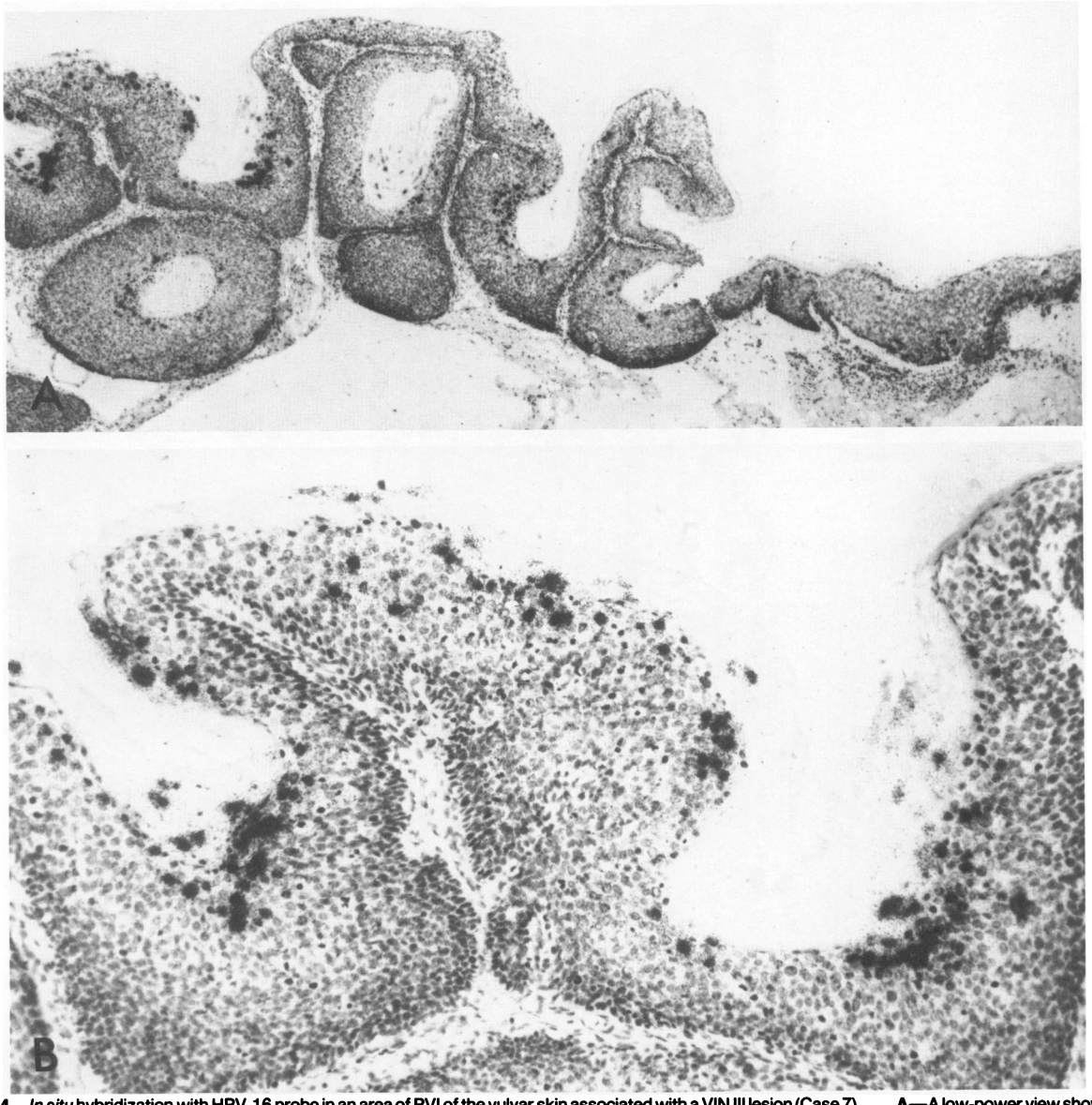

Figure 4—*In situ* hybridization with HPV-16 probe in an area of PVI of the vulvar skin associated with a VIN III lesion (Case 7). **A**—A low-power view showing multiple foci of HPV-16 positive cells. (×44) **B**—A higher magnification showing the superficial location of the positive cells. (×145)

and others showing PVI. HPV DNA was identified at a single site in 17 patients and in more than one site in 13 patients. In 10 of these 13 cases, the same HPV type was identified at all sites of an individual patient. Two tissues (vulva, Case 9; cervix, Case 19) showed double infection; and one tissue (vulva, Patient 12) showed triple infection.

The distribution of HPV genotypes in tissues by site and morphologic features of the individual lesion is shown in Table 2. Viral DNA was detected in 59% of the tissues which had neoplasia with CE and in 58% of the tissues with PVI. HPV-6/11, HPV-16, and HPV-18 were identified, respectively, in 25, 24, and 2 tissues. The cervix was positive more often than the other sites. For lesions of similar type, the distribution of HPV types at different sites was comparable. However, there was a marked difference in distribution of HPV types in lesions with different pathologic features. HPV-16 was associated with 17 of 20 (85%) DNA-positive tissues which had neoplasia with CE but with only 5 of 25 (20%) DNA-positive tissues which had only PVI. The pattern of HPV-6/11 distribution was the reverse of that of HPV-16. HPV-6/11 was associated with 25% of DNA-positive tissues

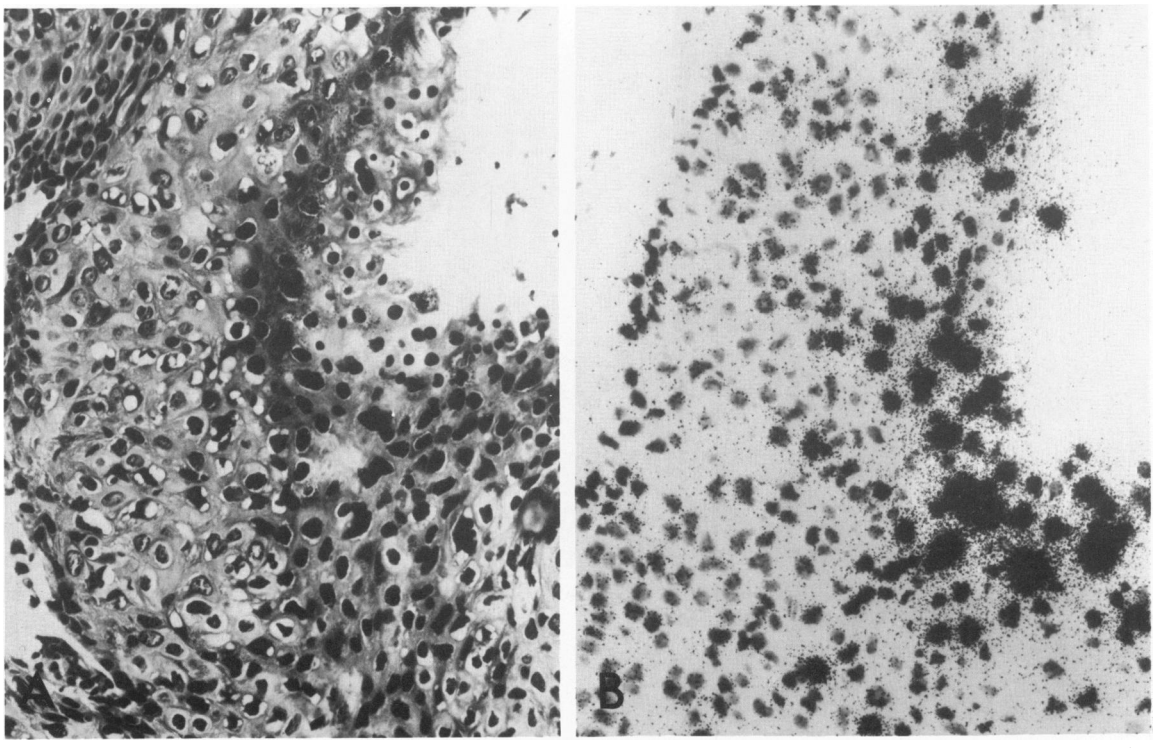

Figure 5—PVI of the uterine cervix synchronous with early vulvar neoplasia (Case 3). **A**—An H & E-stained section. (×240) **B**—A serial section hybridized with an HPV-16 probe, showing strong positivity of the dyskeratocytes and koilocytes. (×240)

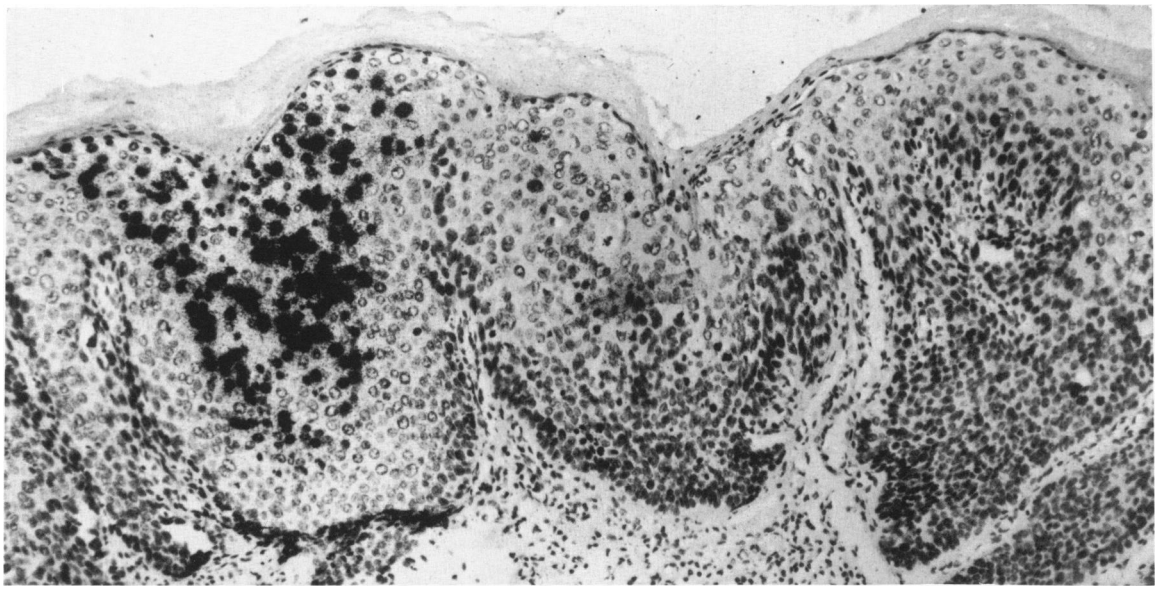

Figure 6—Gamut of PV-associated intraepithelial lesions of the vulvar epithelium (Case 7). Hybridization with HPV-16 probe; hematoxylin counterstain. Discrete focus of positivity in an area of PVI (*left*) adjacent to VIN II (*center*) and VIN III (*right*), which are negative. (×120)

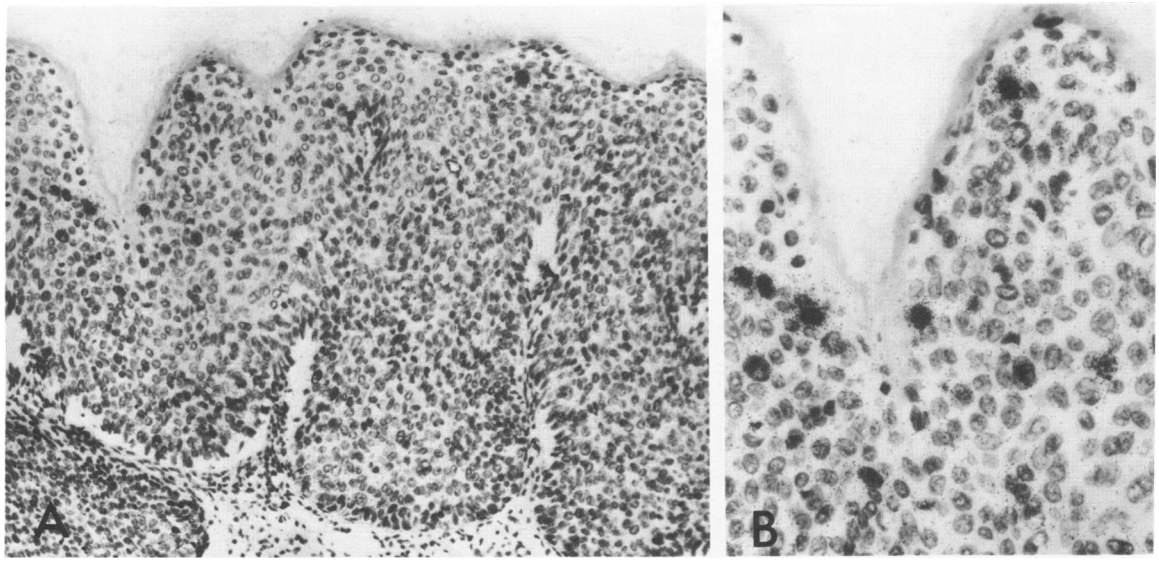

Figure 7—Area of PV-associated VIN II (Case 7) hybridized with HPV-16 probe. **A**—Scattered strongly positive cells in the upper third of the epithelium. (×220) **B**—A higher power view of one positive area. (×430)

Table 1—Distribution of HPV Genotype by Site and Pathologic Features of the Lesion in Virus-Positive Cases

		Pathologic diagnosis								
		Neoplasia				PVI				
Case*	Age	Vulva	Vagina	Cervix	Perineum	Vulva	Vagina	Cervix	Perineum	Other sites
1	66	16		Neg						
2	61	16								
3	41	16		16	NT					
4	34	16		16			6,11		NT	
5	34	6/11		6/11					NT	6/11 (anus)
6	22	16						NT		
7	42	16			16					
8	48	16			NT					
9	43	6/11,16	16							
10	37	Neg		16						
11	42	16								
12	39	6/11,16,18								
13	44			6/11		NT		6/11	NT	
14	32			16		Neg	Neg	Neg		
15	24			16						
16	33			16						
17	22			16		6/11				
18	21					16		16		6/11 (anal canal)
19	26					Neg		16,18		
20	22					6/11	NT	6/11	NT	NT (perineal)
21	36					6/11	6/11	6/11		
22	20					16	Neg	Neg		
23	54					6/11	NT		NT	NT (urethra)
24	23					NT	NT	6/11	NT	
25	43					NT	6/11	Neg		6/11 (perianal)
26	34					NT	Neg	16		
27	21						NT	6/11		NT (urethra)
28	40						6/11	6/11		
29	21						6/11	6/11		
30	59					6/11				

Neg, negative; NT, site involved but not tested.
*Cases 1 and 2 are in Group I, Cases 3–17 in Group II, and Cases 18–30 in Group III.

Table 2—Distribution of HPV Genotypes in Individual Lesions by Site and Morphology of the Lesion

Pathologic diagnosis	Site	Number of tissues		Distribution of genotypes		
		Tested	Positive (%)	6/11	16	18
Neoplasia	Vulva	6	2	0	2	0
	Cervix	2	0	0	0	0
	Subtotal	8	2	0	2	0
Neoplasia with CE	Vulva	20	9 (45)*	3	8	1
	Cervix	10	9 (90)	2	7	0
	Other†	4	2 (50)	0	2	0
	Subtotal	34	20 (59)	5	17	1
PVI	Vulva	14	7 (50)	5	2	0
	Cervix	16	10 (62)‡	7	3	1
	Other†	13	8 (61)	8	0	0
	Subtotal	43	25 (58)	20	5	1
Total		85	47 (55)	25	24	2

*Includes one tissue with a double infection and one tissue with a triple infection.
†Includes vagina, anal canal, perianal skin, and perineal skin.
‡Includes one tissue with a double infection.

which showed neoplasia with CE but with 80% of DNA-positive tissues which displayed PVI.

An additional feature supportive of the role of HPV-16 in neoplasias was the distribution of HPV types in neoplastic cells. HPV DNA was localized in neoplastic cells in 14 tissues; the genome was identified as HPV-16 in 13 instances and as HPV-6/11 in 1 case (Patient 5).

Antigen Detection by HPV Type and Pathologic Features of the Lesion

The capsid antigen was detected in about half of the tissues, almost always in areas showing viral cytopathologic features. It was not detected in tissues showing only neoplasia, but was found at about equal frequency in tissues that showed neoplasia with CE (62%) or PVI (51%) (Table 3). The antigen detection rates in tissues which were infected with HPV-6/11 and HPV-16 were, respectively, 83% and 62%. This difference was not statistically significant. There were eight tissues which were antigen-positive and DNA-negative; they probably represented infection with HPVs other than HPV-6/11, HPV-16 and HPV-18. Thirteen tissues were DNA-positive and antigen-negative.

Discussion

The major objective of this investigation was to determine whether neoplasia in the lower genital tract, especially in the vulva, is associated with any specific HPV types. Pilotti et al[8] observed that vulvar neoplasias frequently have areas which show viral cy-

Table 3—Correlation of Antigen and DNA Detection in Individual Lesions by HPV Type and Pathologic Features

HPV type detected	Number antigens positive/Number tested Pathological diagnosis			
	Neoplasia	Neoplasia with CE	PVI	Total
6/11	0/0	3/3	16/20	19/23 (83%)
16	0/2	10/15	3/4	13/21 (62%)
18	0/0	0/0	0/0	0/0
Multiple	0/0	2/2*	1/1†	3/3
None	0/6	6/14	2/18	8/38
Total	0/8	21/34 (62%)	22/43 (51%)	43/85 (50%)

*One double infection of HPV-16 in neoplastic cells and HPV-6/11 in PVI, both areas antigen-positive. One triple infection of HPV-6/11, 16, and 18, all in adjacent antigen-positive areas of PVI.
†One double infection of HPV-6/11 and 16 in the same antigen-positive area of PVI.

topathic effects and that these areas contain papillomavirus particles and capsid antigen. We utilized the *in situ* DNA hybridization test to identify HPV types in these and other morphologically similar lesions as well as in a comparison group of tissues which showed only PVI without any evidence of associated neoplasia. The results indicate that HPV-16 is strongly associated with neoplasia. It was detected in 85% of the neoplasia with CE lesions but in only 20% of the PVI lesions. The association of HPV-16 with neoplasia was even more striking when one considered the distribution of HPV types in neoplastic cells. In 14 instances the viral DNA was localized in the neoplastic cells; in all but 1 case it was HPV-16 DNA. The HPV-6/11 DNA was distributed in a pattern almost exactly opposite to that of HPV-16. It was only once detected in neoplastic cells. However, HPV-6/11 was the only virus recovered from several sites in one of the most severely affected cases. These data indicate that the patterns of association of HPV-6/11 and HPV-16 in vulvar neoplasia are very similar to those in cervical neoplasia. HPV-18 DNA was identified only twice in the tissues; therefore, its relationship to neoplasia could not be ascertained.

HPV-16 is strongly associated with severe atypias and invasive cancers in the lower genital tract.[2,18,19] These lesions are generally negative for viral capsid antigen.[17] Our results indicate that the morphologic spectrum of HPV-16 infection includes lesions which look like ordinary HPV infection and that in these areas, HPV-16 capsid antigen is readily detected. In lesions of similar morphology, there was no significant difference in the antigen-detection rates between HPV-6/11 and HPV-16. The findings suggest that whereas both HPV-6/11 and HPV-16 infections produce viral cytopathic effect, HPV-16 infections are more likely to progress to neoplasia.

The study also characterized some aspects of the multicentric disease which is seen more commonly now than in previous years.[20] In a majority of the instances, the multicentric disease was caused by the same virus infecting different sites in an individual, but in 3 of 13 cases in which DNA was identified at more than one site, more than one HPV type was detected at different sites in the genital tract. Multiple infection at the same site was found in three of the 47 DNA-positive tissues examined.

The *in situ* DNA hybridization test of routinely collected and pathologically well-characterized material provided valuable information in this investigation. However, the test has several limitations. Crum et al[21] found that the sensitivity of detection of the *in situ* test using a biotinylated SV40 probe was about 800 copies/cell. We have not estimated the sensitivity of our detection method by comparison with tests of DNA extracted from uniformly infected cells; but in our experience, the ^{35}S-labeled DNA probes are able to identify viruses in paraffin sections of tissues by *in situ* tests when the copy number (estimated by Southern transfer hybridization of DNA extracted from fresh unfixed tissue) is an average of 100 or greater. This relative lack of sensitivity probably accounts for our inability to detect viral DNA consistently in invasive cancers, where the cells may contain only a few copies of integrated viral genome. The low sensitivity may also result in underdiagnosis of HPVs which replicate less efficiently and produce low copy numbers. Second, we employed only three HPV probes, although several additional HPVs (HPV-31, 33, 35, and 42) are known to infect the genital tract. The eight antigen-positive DNA-negative tissues probably represent infection with HPVs other than the ones used as probes. Third, closely related HPV types cross-hybridize to some degree even under stringent conditions, and it may not be possible to discriminate between infections by two closely related types (eg, HPV-6 and HPV-11 and between HPV-16 and HPV-31) unless the tissue is screened separately with each probe. We did not attempt to discriminate between HPV-6 and HPV-11 and have found that under our test conditions, cross-hybridization between HPV-16 and HPV-31 is infrequent and minimal.

Results from other systems indicate that the *in situ* hybridization test can be refined to detect single copies of small chromosomal genes, and it is anticipated that the *in situ* tests for HPV will be further refined to attain greater sensitivity and be made more specific by employing subgenomic monospecific probes.[12] Further, molecular analysis of HPV-associated pathologic tissues may be expected to contribute significantly to the understanding of the pathogenesis of these lesions.

References

1. Richart RM: Cervical intraepithelial neoplasia: A review. Pathol Annu 1973, 8:301–328
2. 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 1983, 80:3812–3815
3. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H: A new type of papillomavirus DNA: Its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J 1984, 3:1151–1157
4. Crum CP, Fu YS, Levine RU, Richart RM, Townsend DE, Fenoglio CM: Intraepithelial squamous lesions of

the vulva: Biologic and histologic criteria for the distinction of condylomas from vulvar intraepithelial neoplasia. Am J Obstet Gynecol 1982, 144:77–83
5. Crum CP: Vulvar intraepithelial neoplasia: The concept and its application. Hum Pathol 1982, 13(3):187–189
6. Rastkar G, Okagaki T, Twiggs LB, Clark BA: Early invasive and in situ warty carcinoma of the vulva: Clinical, histologic, and electron microscopic study with particular reference to viral association. Am J Obstet Gynecol 1982, 143:814–820
7. Crum CP, Braun LA, Shah KV, Fu YS, Levine RU, Fenoglio CM, Richart RM, Townsend DE: Vulvar intraepithelial neoplasia: Correlation of nuclear DNA content and the presence of a human papillomavirus (HPV) structural antigen. Cancer 1982, 49:468–471
8. Pilotti S, Rilke F, Shah KV, Delle Torre G, De Palo G: Immunohistochemical and ultrastructural evidence of papillomavirus infection associated with in situ and microinvasive squamous cell carcinoma of the vulva. Am J Surg Pathol 1984, 8:751–761
9. Mathieu A, Avril MP, Duvillard P, George M, Orth G, Giou G, Prade M, Gerbaulet A, Wolff JP, Michel G: [Buschke-Lowenstein tumor: 3 vulvar localizations: Association with HPV-6 in one case.] Ann Dermatol Venereal 1985, 112:745–746
10. Beckmann AM, Myerson D, Daling JR, Kiviat NB, Fenoglio CM, McDougall JK: Detection and localization of human papillomavirus DNA in carcinomas by in situ hybridization with biotinylated probes. J Med Virol 1985, 16:265–273
11. Gupta J, Gendelman HE, Naghashfar Z, Gupta P, Rosenshein N, Sawada E, Woodruff JD, Shah K: Specific identification of human papillomavirus type in cervical smears and paraffin sections by *in situ* hybridization with radioactive probes: A preliminary communication. Int J Gynecol Pathol 1985, 4:211–218
12. Stoler MH, Broker TR: In situ hybridization detection of human papillomavirus DNAs and messenger RNAs in genital condylomas and a cervical carcinoma. Hum Pathol 1986, 17:1250–1258
13. Ostrow R, Manias D, Clark B, Okagaki T, Twiggs L, Faras A: The analysis of malignant tumors of the vulva, vagina and cervix for HPV DNA (Abstr). Fifth International Workshop, The Papillomaviruses, Cold Spring Harbor, New York, September 2–7, 1986
14. Gissmann L, Diehl V, Schultz-Coulon HJ, zur Hausen H: Molecular cloning and characterization of human papillomavirus DNA derived from a laryngeal papilloma. J Virol 1982, 44:393–400
15. Gissmann L, Wolnik L, Ikenberg H, Koldovsky U, Schnurch H, zur Hausen H: Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc Natl Acad Sci USA 1983, 80:560–563
16. Brahic M, Haase A: Detection of viral sequences of low reiteration frequency by in situ hybridization. Proc Natl Acad Sci USA 1978, 75:6125–6129
17. Gupta JW, Gupta PK, Shah KV, Kelly DP: Distribution of human papillomavirus antigen in cervical vaginal smears and cervical tissues. Int J Gynecol Pathol 1983, 2:160–170
18. Crum CP, Ikenberg H, Richart RM, Gissman L: Human papillomavirus type 16 and early cervical neoplasia. N Engl J Med 1984, 310:880–883
19. Crum CP, Mitao M, Levine RU, Silverstein S: Cervical papillomaviruses segregate within morphologically distinct precancerous lesions. J Virol 1985, 54:675–681
20. Okagaki T: Female genital tumors associated with human papillomavirus infection, and the concept of genital neoplasm-papilloma syndrome (GENPS). Pathol Annu 1984, 19(Part 2):31–62
21. Crum CP, Nagai N, Levine RU, Silverstein S: In situ hybridization analysis of HPV 16 DNA sequences in early cervical neoplasia. Am J Pathol 1986, 123:174–182