CLINICAL AND SEROLOGICAL MANIFESTATIONS OF GENITAL HUMAN PAPILLOMAVIRUS INFECTION

by

Arne Wikström

Department of Dermatovenereology, Karolinska Hospital, Stockholm, Sweden

and

Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, Sweden

Stockholm 1995

SUMMARY

Efficacy of chemical and/or surgical treatment for penile and anal condylomata acuminata was investigated in two retrospective studies of hetero- and homosexual men. Variation in clinical features and symptomatology as well as the reliability of diagnostic criteria by different methods for acetowhite penile lesions was also studied. Furthermore, the antibody response in the course of penile wart disease as well as in asymptomatic genitoanal papillomavirus infection (GPVI) was analysed.

In the first retrospective study, as much as 23% of patients still had condylomas after one year of chemical and/or surgical treatment. On the other hand, 38% were cured after a single treatment session. In the group mainly with anal warts, concurrent penile warts were significantly more common among heterosexual men compared to homosexual men ($p<0.001$), while intra-anal wart growth was more common among the homosexual males ($p<0.001$). When comparing diagnostic methods for subclinical penile HPV infection, conventional histopathology appeared to be the most valuable diagnostic aid to penoscopy, while the additional use of Southern blot, in situ hybridisation and PCR assays for HPV DNA detection did not increase the predictive value of GPVI. We also describe a new distinct clinical entity, HPV-associated balanoposthitis, comprising a wide range of often long-lasting symptoms, such as itching, burning and dyspareunia.

A significant increase in the IgG antibody response against defined epitopes in the L1 and L2 capsid proteins of HPV 6, was found among men with previous condylomata. By following a cohort of STD clinic patients with multiple brush samples from the genitoanal region as well as serum samples taken at several consecutive clinical visits, we identified 16 patients who had seroconverted to HPV seropositivity during follow-up. Antibody responses to several HPV-derived peptide and protein antigens were induced at the same time. Seroconversions were usually seen concomitantly with HPV acquisition or at the visit after HPV DNA was first detected. The HPV antibody response was frequently transient and declined or disappeared after clearance of infection. The antibody responses were induced by several different HPV types, indicating limited type-specificity. The most type-restricted response was against HPV 16 capsids, where seroconversions to continuous seropositivity were induced by infection with HPV 16.

Key words: condyloma; genital warts; synthetic peptide; ELISA

ISBN 91-628-1542-3

To my parents

CONTENTS

ABBREVIATIONS

AIN	anal intraepithelial neoplasia
b p	base-pair
CIN	cervical intraepithelial neoplasia
CIS	carcinoma in situ
DNCB	2.4-dinitrochlorbenzene
ELISA	enzyme-linked immunosorbent assay
GPVI	genitoanal papillomavirus infection
HPV	human papillomavirus
HSV	herpes simplex virus
ISH	in situ hybridisation
LC	Langerhans cell
LCR	long control region
MHC	major histocompatibility complex
NCR	non-coding region
ORF	open reading frame
PCR	polymerase chain reaction
PIN	penile intraepithelial neoplasia
RR	relative risk
SB	Southern blot
SCC	squamous cell carcinoma
STD	sexually transmitted disease
VIN	vulvar intraepithelial neoplasia

ORIGINAL PAPERS

This thesis is based on the following articles, which will be referred to in the text by their Roman numerals:

I. von Krogh G, Wikström A.
Efficacy of chemical and/or surgical therapy against condylomata acuminata: A retrospective evaluation.
Int. J. STD & AIDS 1991;2:333-338

II. Wikström A, Hedblad M-A, Johansson B, Kalantari M, Syrjänen S, Lindberg M, von Krogh G.
The acetic acid test in evaluation of subclinical genital papillomavirus infection: A comparative study on penoscopy, histopathology, virology and scanning electron microscopy findings.
Genitourin. Med. 1992;68:90-99

III. Wikström A, von Krogh G, Hedblad M-A, Syrjänen S.
Papillomavirus-associated balanoposthitis.
Genitourin. Med. 1994;70:175-181

IV. von Krogh G, Wikström A, Syrjänen K, Syrjänen S.
Anal and penile condylomas in HIV-negative and HIV-positive men; clinical characterization, results from in situ HPV DNA hybridisation, and long-term therapeutic endavour.
Manuscript

V. Wikström A, Eklund C, von Krogh G, Lidbrink P, Dillner J.
Levels of immunoglobulin G antibodies against defined epitopes of the L1 and L2 capsid proteins of human papillomavirus type 6 are elevated in men with a history of condylomata acuminata.
J. Clin. Microbiol. 1992;30:1795-1800

VI. Wikström A, van Doornum G, Quint W, Schiller J, Dillner J.
Identification of human papillomavirus seroconversions.
J. Gen. Virol, In press

VII. Wikström A, van Doornum G, Kirnbauer R, Quint W, Dillner J.
A prospective study on the development of antibodies against human papillomavirus type 6 among patients with condyloma acuminata or new asymptomatic infection
Manuscript

PAPILLOMAVIRUSES

General properties, classification

Papillomaviruses, belonging to the Papovaviridae family, consist of small (55 nm in diameter), non-enveloped viruses with 72 capsomers in a capsid with icosahedral symmetry. The genome consists of a circular, double-stranded DNA molecule of about 8000 base pairs (bp). Only one DNA strand is coding, i.e. the genome is only transcribed in one direction (284). The papillomavirus genome contains a number of open reading frames (ORFs), which can be translated into a protein. The ORFs are subdivided into the early region ORFs (numbered E1-E7) which are required for transformation and episomal maintenance, and the late region ORFs (numbered L1-L2) which code for the capsid proteins.

Seventy different HPV types have been identified so far, of which 34 have been found in genitoanal lesions (72). Initially, the definition of a new type was a virus isolate that exhibited less than 50% cross-hybridisation in liquid hybridisation to any known HPVs (71). Since the E6, E7 and L1 ORFs of new viral isolates are now routinely sequenced, a new definition has been established. If the nucleotide homology in these ORFs is lower than 90% to any known HPV type, the new isolate will be defined as a new type (72).

The HPV types have been classified as "cutaneous" or "genital" types, according to the location from which they initially were isolated (72); in Figure 1, a "phylogenetic tree" is shown (modified from van Ranst et al (328)). The types associated with genital intraepithelial dysplasia and cancer, are referred to as "high-risk" types, and those found in benign genital warts (condylomata acuminata), as "low-risk" types. The cutaneous types are subdivided into the types causing benign warts such as "verruca vulgaris", plane warts and inverted warts, and those being found almost exclusively in the lesions of patients with the rare hereditary skin disorder "epidermodysplasia verruciformis". The latter HPV types have also been found in skin cancers among allograft recipients, such as patients receiving renal transplants (71).

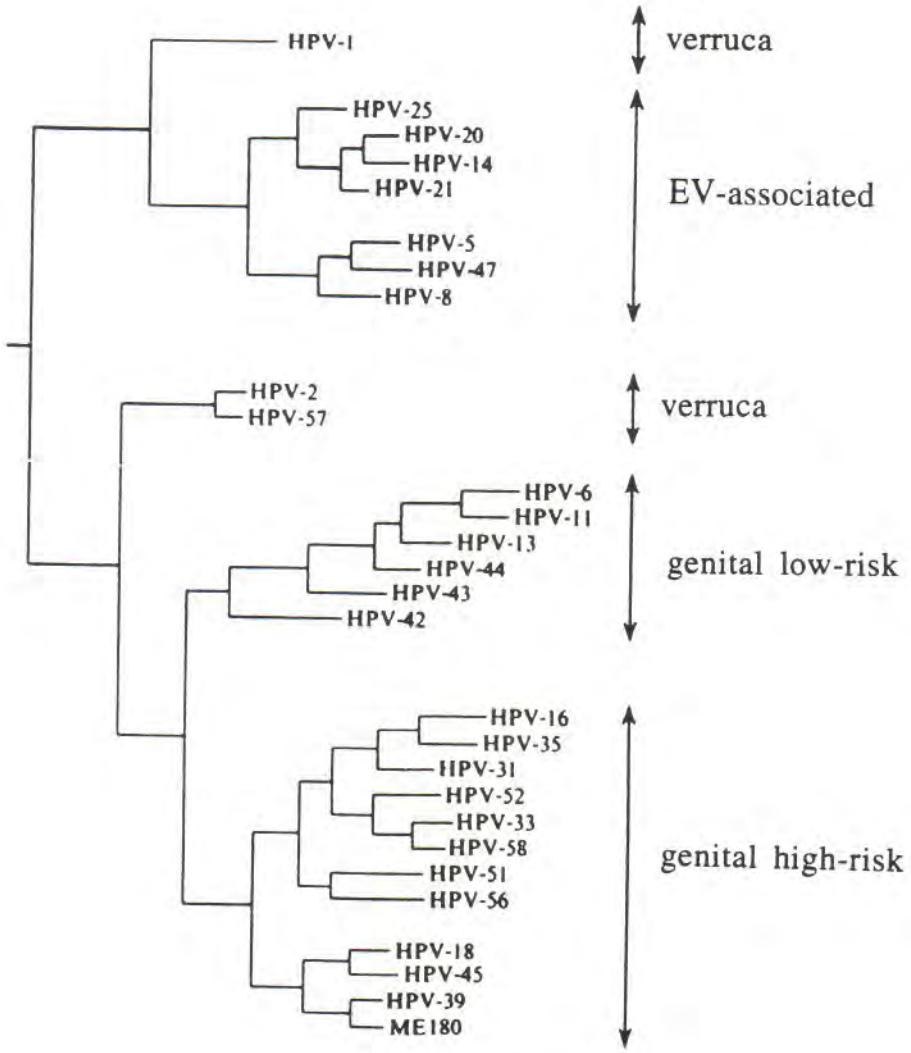

Fig. 1. Phylogenetic tree based on the degree of nucleotide homology between various HPV types. The clinical manifestations of the HPV types are inserted to the right.

HISTORY

Condylomata acuminata, or the classical genital warts have been known since ancient times (10). Greek physicians first discribed the disorder, and used the term "condyloma" (rounded tumour); it was considered to be of venereal origin, and mostly transmitted by anal intercourse among men (10). Roman physicians discussed the etiology of condylomas, and the disease also appears in old Roman poetry (10). Other terms, such as "Thymus" (due to the resemblance of the plant Thymus capitatus and condylomata) and "Ficus" (a more obscene word, probably used due to the similarity of the surface of the plant Ficus sycomorus and condylomata, a word that still has survived and is still being used in German as "Feigwarze"). During the "Dark ages" the issue of venereal diseases rather became one of quiescence, perhaps even oblivion. After the arrival of syphilis to Europe at the end of the 15th century (350), however, a renewed interest in venereal diseases arose. Nevertheless, at this point of history no pathogenetic and clinical distinction was made between syphilitic, gonorrhoeal and genital wart manifestations, all being regarded as a manifestation of syphilis (some of the specific lesions in secondary syphilis are still referred to as "condyloma lata") (230). In 1793, Benjamin Bell reported that he regarded syphilis and gonorrhoea as disconnected disorders (19). At the same time, an up-dated description of condylomas was made. At the end of the 19th century, Gemy discussed the similarities between genital warts and skin warts (110). Cutaneous warts were one of the first diseases where viral etiology could be proven by experimental inoculation of filtrates from verruca vulgaris (56). The viral particles were first visualised by electron microscopy using extracts of skin warts (297).

TROPISM

Papillomaviruses are common among a wide range of different species, including man as well as other mammals such as monkeys, dogs, sheep, cattle, and also fish and birds (302). The papillomaviruses are principally species specific, each infecting only its natural host, e.g. the HPVs do not infect other species than

humans (284). The productive cycle of the HPVs is dependent on the differentiation of the infected epithelial cells (18). Most papillomaviruses, like HPVs, only infect the epithelial cells (keratinocytes), except for some animal papillomaviruses that are also able to infect dermal fibroblasts (284).

VIRAL PROTEINS

E1

E1 is the longest and the second most conserved ORF. The full-length E1 ORF codes for a nuclear DNA-binding phosphoprotein which is responsible for maintenance of the episomal form of the viral DNA (187, 188). The E1 protein complexes with the E2 protein to form an initiation complex which binds to the viral origin of replication (269).

E2

The full length E2 protein acts as a transcriptional transactivator when it binds to the E2-responsive elements (E2-RE) located in the LCR (316). However, when E2 binds to E2-binding sites close to the TATA-box of the E6/E7 promoter it can instead act as a transcriptional repressor of E6/E7 expression (25, 317). Integration of the viral genome into the cellular DNA may lead to a disruption of the E1 or E2 ORF (12), which in turn may lead to loss of the negative regulatory control exerted by the E2 protein and result in an increased expression of E6 and E7 proteins (61). E2 is also required for viral replication, a function mediated by the E1-E2 complex that binds the viral origin of replication (54).

E4

The E4 protein, is expressed late in the viral productive cycle and is primarily located in the cytoplasm (85). The E4 protein disrupts the cytoskeleton of the host cell (86), resulting in the typical "hollow" morphologic appearence of productively HPV-infected cells, known

as koilocytes. The cytoskeleton disruption is thought to facilitate release of viral particles (86).

E5

The E5 protein in BPV 1 is hydrophobic, only 44 amino acids long, and is the main in vitro transforming protein for BPV (271). The E5 gene of HPV 16 can transform human keratinocytes (181). The major mechanism of transformation in BPV is via activation of the platelet-derived growth factor (PDGF) receptor (239).

E6

The HPV E6 protein is required for transformation, and HPV 16 E6 can also transform primary human fibroblasts by itself (349). E6 of HPV 16 and 18 complex with the cellular p53 protein and induce accelerated degradation of p53 (268, 352), which normally functions as a tumour suppressor protein (102). An inverse correlation has been found between the presence of HPV and p53 mutations in cervical tumors; HPV positive cervical cancer cell lines harbour wild-type p53, whereas HPV-negative cervical cancer cell lines contain mutated p53 (62, 355), although recent studies analysing large numbers of cervical cancer biospies did not observe this pattern (315).

E7

E7 is the major transforming protein of HPV. In collaboration with activated ras, it is able to achieve malignant transformation of rodent cells (198). Like adenovirus E1A protein and SV 40 T-antigen, the E7 protein is capable of binding to the retinoblastoma (RB) protein (89). In this way it prevents the normal interaction between RB and the cellular transcription factor E2F, which in a free form is capable of transciptional transactivation in the S phase of the cell cycle (51). Similarly, it has been demonstrated that the cellular protein p107 can bind to E2F. HPV E7 also can bind to the protein p107 reducing the E2F-p107 binding, and thus prevent inactivation of E2F through the S phase (138). E7 of HPV 16 binds

to RB with 5-20 times higher affinity than does the E7 of HPV 6 and 11 (218).

L1

The L1 is the most conserved ORF in papillomaviruses and has approximately 40% amino acid homology also between distantly related HPV types (12). It codes for the major capsid protein, which has a molecular mass of about 57 kDa (183).

L2

This ORF codes for the minor capsid protein, which together with the L1 protein forms the capsid of the virion (159).

LCR

The long-control region (LCR), also termed the non-coding region (NCR), is an intermediate DNA stretch located between L1 and E6. This part of the viral genome has no protein-encoding capacity, but contains signals for the control of transcription as well as for replication of the virus. The activity of this region is dependent on cellular transcription factors, like NF1, SP1, AP1 and the glucocorticoid receptor (316).

DIAGNOSTIC METHODS

HPV typing assays

Southern Blot hybridisation

This technique has been viewed as the "golden standard" among HPV DNA detection methods with a sensitivity range between 0.1 and 0.01 viral copies per cell, but is too time consuming and labour intensive for routine diagnosis (73). After extraction of cellular DNA from a specimen, it is digested with a restriction enzyme and the fragments are separated by electrophoresis on a gel. Subsequently, the DNA is denatured, neutralised and transferred to a filter, which is then subjected to hybridisation with labelled HPV probes. If the

filter is hybridised with a mixture of probes under low stringency conditions a broad range of HPV DNA types can be detected, whereas high stringency conditions are used for HPV DNA typing.

In situ hybridisation

As this technique is applied to tissue sections, it is possible to determine the localization of the HPV DNA within the specimen. Both radioactive (^{35}S-labeled) probes or non-radioactive probes, such as biotinylated or digoxigenin-labeled DNA probes can be used. The radio-labeled probes can detect 20-50 viral genomes per cell (274), whereas the sensitivity of biotinylated probes can vary from as low as 800 genomes per cell (63) to sensitivities equal to or better than ^{35}S-labeled probes (306). The biotinylated probes are more stable than the radioactive probes, and do not expose the staff to radioactivity.

Polymerase chain reaction (PCR)

The PCR technique has revolutionised the virological field due to its extremely high sensitivity, being able to detect one viral genome in 100.000 cells (73). After the repetitive cycles with denaturation/primer annealing/primer extension, the sample is separated by gel-electrophoresis, and finally hybridised to HPV probes. Alternatively, PCR-products can be digested with restriction enzymes to characterise the HPV type present. Since a large number of primers are available, the choice of primer is crucial. Most of the consensus primers are derived from the well conserved E1 or L1 regions (324). In case of integrated DNA, primers from the E6 or E7 regions would be preferrable; these ORFs are unfortunately among the least conserved and are not suitable for the generation of general primers, although some such primers exist (324). The consensus primers allow amplification of a range of known and unknown HPV types for later characterisation of the HPV type. PCR using type specific primers is, however, usually more sensitive than PCR using consensus primers (236). PCR can also be used on paraffin embedded sections (224).

When the PCR technique was first applied to detect HPV, there were severe problems with contamination, resulting in false positive results. These difficulties can, however, be reduced substantially by following strict clinical and laboratory procedures (118). The technique is now widely used and has become the preferred method for HPV investigations. Previous prevalence studies using easy and cheap techniques, such as filter in situ hybridisation and dot blot, have had severe problems with reproducibility (162). On the other hand, various PCR studies reporting presence of HPV DNA at unusual sites are more trustworthy if they are also confirmed by less sensitive methods.

Cytology/histopathology

An acanthotic, hyper- and parakeratotic epidermis associated with varying degrees of hyperplasia is generally seen in HPV-induced lesions by light microscopy. Only the presence of koilocytosis (160) is pathognomonic for HPV infection. The koilocyte represents the pathognomonic hallmark of replicative HPV infection, appearing as an epithelial cell presenting with an irregular, hyperchromatic nucleus surrounded by a cytoplasmatic halo (160). Correlating these five histopathologic criteria, Rock et al (257) examined biopsies from clinically typical genital warts with histology and in situ hybridisation. Although 88% of the samples were HPV-positive, only 71% fulfilled at least three of five histopathological criteria, indicating that a risk of false negative histology exists. This has been further elucidated by the fact that it is in no way uncommon that lesions being HPV positive by highly sensitive PCR assays lack the presence of koilocytosis (223). Yet, in order to increase the sensitivity, the in situ hybridisation method still represents a valuable complement to conventional histopathology. (44).

The degree of intraepithelial neoplasia (IN) is graded I, II or III depending on the extent of dysplastic involvement of the epithelium. The genitoanal site is also given, CIN, VIN, VAIN, PIN, PEIN or AIN are used if cervix, vulva, vagina, penis, perineum or anus is afflicted, respectively. Koilocytotic cells are mainly found in

condylomatous lesions and in low-grade dysplasias and are usually absent in high-grade lesions and in cancers (348).

The Papanicolau (Pap) smear, described in the 40ies (235), has been used as a cytological screening method for cervical cancer and CIN in most Western countries (in Sweden since the 60ies) (242). The rationale for these screening programs is based on the observation that early detection of dysplasia and cancer in early stages significantly reduces the incidence of invasive disease and of mortality from invasive cancer (210), and a decreased incidence and mortality from cervical cancer has also been observed in many countries where large-scale screening programs have been performed. In some studies the rate of invasive cancer has dropped up to 70% (294, 325). In Norway - in contrast to Sweden, Finland and Iceland - where the screening program until recently was not organised systematically, no significant change in the cancer incidence was seen. Available data stress the importance of implementing an organised screening program with a high coverage of the population at risk (291), as being evident from case-control studies demonstrating that women attending cytological screening programs have a strongly reduced risk of cervical cancer. The protective effect is proportional to the number of previous screening occasions (68), and it appears that the most important factor for efficacy of cervical screening programs is the implimentation of organised programs that ensure that as many women as possible are being screened. Nevertheless, some inherent weaknesses exist for the current Pap smear programs. Thus, the test is associated with a false negativity rate of 20-25% (202, 209), which inevitably may confer a false confidence to the screened women. Quality control efforts to ensure that the samples are taken correctly and that the cytological slides are correctly interpreted are also important (210). Accordingly, the efficacy of general cytology screening is dependent on three major factors: high attendance rate of the population, the skill of the medical personnel for taking the sample and the quality of the laboratory for correct interpretation (210).

The acetic acid test

Application of a solution of 5% acetic acid on the genital epithelium will cause a whitish colour due to a swelling of infected suprabasal cells, mainly due to overexpression of cytokeratin 10 (190). The test helps to delineate the lesion, in particular in the diagnosis of a subclinical infection, that is not visible without the aid of acetic acid. The test is rather sensitive, but is associated with some degree of false positivity; inflammatory disorders, for example, may also cause acetowhitening ((16, 279, 314, 327); paper II).

Colposcopy

Since decades, magnification of the cervical area under colposcopic guideline, has been an important tool in the diagnosis of pre-neoplastic and neoplastic lesions (342). In recent years, the technique has also been used to visualise GPVI lesions in general ((131, 169, 279, 281); paper II). Nevertheless, investigations on specific criteria for conspicuous and typical HPV induced lesions have been sparse (251) and have not been accounted for in any detail in GPVI afflicted men, although the term "penoscopy" has been used for the method when applied for investigation of the penis (paper II). One major advantage is that, under colposcopic magnification, biopsies are more easily taken from representative areas, and the instrument is also advantageous for delineation of the afflicted area during therapy.

GROSS APPEARANCE AND ANATOMICAL DISTRIBUTION

Clinically, a coarse distinction is usually made between three morphologic types of lesions; acuminate and papular warts as well as flat lesions. As a common denominator all lesional types are often referred to within the modern term genitoanal papillomavirus infection (GPVI) (342). Acuminate warts are usually caused by HPV 6/11 (309), while papular and flat lesions may also contain the oncogenic HPV types that are rarely found in acuminate lesions (16, 189). More than one lesional type often occur concurrently in individual patients. Classical GPVI manifestations, generally denominated "condylomata" or "condylomas", are papilliferous with

finger-like peduncles exhibiting a high grade of vascularization (342). Acuminate warts are most frequently found in moist areas, such as the preputial cavity (i.e. the glans penis, the coronal sulcus, the fraenulum and the inner aspect of the foreskin) and the meatus urethra in men. In females, these warts are most commonly located on the labia minora, the inner parts of the labia majora, the clitoris, the introitus, the vagina and the cervix. Besides on such moist intertriginous areas, acuminate warts might also be found on dry, keratinized genital and peri-genital skin, such as the penile shaft, the outer parts of the labia majora and the groins.

In contrast to acuminate warts, papular warts are protuberant lesions exhibiting a relatively smooth rounded surface, being mostly found on dry keratinized genital skin areas such as the penile shaft, the lateral parts of the vulva, the perineum and the perianal area. The colour of the warts are usually brownish on pigmented sites, and on mucosa more pinkish-red. Although not being very protuberant, flat warts are also raised somewhat over the surrounding epithelium and usually exhibit undulating wavy surfaces, sometimes with microspikes. They may be located anywhere on the genital epithelium, are probably more common than acuminate warts and are often overlooked.

Vulvar papillomatosis has been reported to be HPV associated, whereas other authors have reported that these lesions are normal anatomic variants similar to pearly penile papules in men (1). This difference might be due to different definitions of the disease. Probably some "papillomatosis" represents normal anatomic variants, whereas others may be a manifestation of HPV infection (for review, see Sawchuk (265)).

The distribution of condylomata on the penis differs between uncircumcised and circumcised men; in the former the preputial cavity is usually afflicted, but in the circumcised men involvement of the penile shaft is more common (60).

About 10-28% of men with condylomata, have involvement of the distal urethra (229, 336). In females, the corresponding figure is below 5% (55). Bladder involvement is very uncommon, being

reported only in a few cases in the literature (75, 287), and the most extensive cases usually being present among immunosuppressed patients (75). A simple meatoscopy is generally sufficient in the clinical routine to delinate the lesions (318, 319). Warts located proximally of the fossa navicularis are rare. Still, referral to urologist for urethroscopy is indicated in about 5% of cases (55, 229).

Involvement of the perianal area and the anal canal is common in both sexes (230). The dentate line serves as a natural border, and involvement of warts beyond this level is very rare (35). Anal warts in men do not only occur among homosexuals, in fact the coexistence of penile and anal warts in heterosexual men is not uncommon (114).

Besides causing cosmetic disturbances, GPVI can cause local symptoms (such as itching, burning, bleeding and dyspareunia), but may also lead to psychosexual problems, such as blame and hypochondric fears (101). In one study, as much as 40% of men with condylomata reported a negative effect on their sexual life (347). Problems tend to increase in patients afflicted with warts for long periods of time. In an American inquiry (ASHA 1993) (9) among patients with long lasting infection, the majority reported difficulties in starting a new sexual relationship (86%) and decreased enjoyment of sexual life (68%). About 70% of the responders became isolated and less spontaneous.

Acetowhite lesions

The classical warts are less common than latent or subclinical infections (340). In latent infections HPV DNA is detected in the epithelium, without causing any distinctive lesions detectable either by colposcopy or histology (223).

The presence of clinically concealed GPVI lesions was first described on the cervix by Meisels et al (204). The term subclinical HPV infection was first applied to GPVI lesions of the cervix (250), but has later also been used for lesions on the outer genitals that

are largely hidden to naked eye examination (342). Nevertheless, some confusion exists in the nomenclature regarding the differentiation between flat and subclinical lesions, which are used synonymously by some authors. Strictly, however, clinical HPV infections either cause symptoms or are visible to the naked eye. Subclinical lesions, on the other hand, are undiscernible to the eye, but are appreciable by colposcopic magnification and in particular after application of acetic acid. However, use of colposcopic magnification is not always very precise for the diagnosis; the correlation between colposcopy and histology may be poor (42).

Acetowhite lesions may sometimes be symptomatic in both sexes. The condition was first described in women as papillomavirus vulvitis, causing symptoms such as itching, burning, fissures, dryness and dysuria (29, 30), but has later also been described in men and denominated "papillomavirus-associated balanoposthitis" (paper III). Anyhow, in these patients, colposcopically typical acetowhite lesions are detected; when biopsied, HPV specific histological changes are seen.

THERAPY

The improvement of effective treatment against GPVI would be of importance, as some HPV types are associated with premalignant and malignant conditions (240). Unfortunately, no single method of management is universially effective. Recurrence rates following treatment are generally rather high (40, 343). Most therapies, except for podophyllotoxin, are clinic-based, time consuming and protracted (252). At present, treatment is only being recommended for visible condylomata for cosmetic reasons and for flat lesions if they are symptomatic. Among patients with multiple partners, treatment may also be attempted as an epidemiological approach in order to minimize the risk for contagion to the partners (98). Removal of intraepithelial neoplasias is also highly recommended, although the magnitude of the risk for malignification is unknown (226). Management includes chemical and surgical methods.

Chemical treatment

Podophyllin, an alcoholic 20-25% extract of the podophyllum emodi or podophyllum peltatum plant resins, has been used for many years for topical treatment of genital warts since preliminary reports on efficacy were reported in 1944 (65). The most biologically active component is podophyllotoxin, which acts as a cytostatic drug exerting an antimitotic effect by arresting cell division in metaphase and provoking necrosis of condylomata (115). The podophyllotoxin is now available in a 0.5% solution (Wartec^{R}, Condyline^{R}), which is applied twice daily at home by the patient for one or several three-days cycles. In contrast to podophyllin the remedy does not have to be washed off (341). Surplus of podophyllin must otherwise be wiped off 4-6 hours after application in order to minimize a substantial risk of severe local side effects. Another disadvantage of podophyllin compared to podophyllotoxin, is its unreliable stability and highly variable quality regarding biologically active compounds between different batches (336). Efficacy of podophyllotoxin solution for home-treatment is superior to that of podophyllin and the modality is not only more convenient for the patient but may reduce the cost of therapy by decreasing the required number of clinic visits (153, 173, 341). In one study, the long-term cumulative cure rate from 1-2 three-day courses of podophyllotoxin treatment against penile warts was found to be as high as 82%, a significant improvement compared with 1-2 applications of 20% podohyllin (343). Other investigators have reported cure rates ranging between 38-63% and 68-88% for podophyllin and podophyllotoxin, respectivley (90, 173, 199). Efficacy is highest for warts located in the preputial cavity, compared to condylomas on the penile shaft and in the urethral meatus (339). Self-application for vulvar warts in women also appears to be safe and efficacious (13), but the substance should not be used in pregnant women due to its embryotoxic properties. Regarding systemic absorption and safety aspects, the use of 0.5% podophyllotoxin is highly satisfactory, which is not the case for 20% podophyllin (337). Severe systemic effects, particularly neurotoxicity including the development of weakness, polyneuritis, paralytic ileus, coma and death have occassionally been reported for podophyllin (103). Local side effects of

podophyllotoxin occur in about half of the patients and include local cutaneous irritation, which is, nevertheless, generally mild and of short duration (335).

The pyrimidine antagonist 5-fluorouracil (5-FU), acting through inhibition of RNA/DNA synthesis, may also be used topically on genital warts (for review see (341)). Local side effects with painful ulcerations are common. In our country the use has been limited to intrameatal condylomas; in up to 90% of cases the warts may disappear after treatment with the cream after each voiding for 2-3 weeks (341). It has also been used as prophylaxis against recurrences after ablation of warts by other methods (23, 168).

Surgical treatment

In more long-lasting and/or hyperkeratotic genital warts, chemical treatment is generally less successful and surgery is usually needed. Surgical treatment includes simple scissor excision, electrocautery, carbon dioxide laser and cryotherapy. All surgical methods on the outer genitals require more or less local anaesthesia, which can be given as infiltration anaesthesia (lidocaine or prilocaine), or applied as a cream containing a mixture of lidocaine and prilocaine (EMLA^{R}). Carbon dioxide laser treatment has been recommended by many authors (14, 20, 98), in particular because of the endorsed advantages compared to conventional surgery being expressed by some authors (14). In a controlled trial, Duus et al (87) compared laser treatment with electrocautery and found no significant differences in numbers of recurrences, postoperative pain, healing time and rate of scar formation. In fact, treatment of recalcitrant condylomata with laser did not offer any advantages over traditional surgery (87). Success rates between 60% and 90% against genital warts have been reported for various surgical methods, such as laser therapy (11, 100, 164), surgical excision (144) and electrodesiccation (289, 295), with recurrences in about 25% of cases (144, 295). The reports on surgery against condylomas differ from the efficacy against acetowhite lesions where recurrence rates of up to 88% have been accounted for (45, 256).

The smoke produced from carbon dioxide laser therapy has been found to contain HPV DNA (99), and there are anecdotal reports of airway HPV infection induced in laser surgeons (e.g. (120)). Sawchuk et al (266) noted the presence of higher amounts of viral DNA in laser debris as compared with electrocautery debris. Therefore, as an appropriate safety measurement the American College of Obstetricians and Gynecologists currently recommends that all personells in the room where laser is being performed should wear adequate masks (58). On the other hand, some investigators assume that masks mostly are designed to keep respiratory bacteria out of the patient´s surgical wound to inhibit iatrogenic infection, but are worthless in protecting the surgeon from inhaled virus (354). When combining smoke evacuator systems with surgical mask protection during the operation, the risk of transmission of HPV is believed to be minimal (266).

Cryotherapy is often used without anaesthesia and is usually well tolerated by many patients, although others experience severe pain, when local anaesthesia is required. Cryotherapy often has to be performed weekly for several weeks. The greatest advantage of cryotherapy is its usefulness on the cervix (21) and in pregnant women (22), but it is also applicable on the outer genitals and in the anal canal (66). In our country the use of cryotherapy with liquid nitrogen for eradication of genital warts in men is quite uncommon, compared to in many other countries where it represents one of the most commonly used surgical methods (158).

Often, intrameatal warts do not respond to chemical treatment (336), and should rather be eradicated surgically (74). Fear of potentially inducing scar formation has been expressed but should be avoidable whenever depth of surgery does not exceed the very upper part of the dermis.

Cure of subclinical lesions is sometimes achieved after a few surgical treatment sessions, but some cases may be quite recalcitrant and signify severe clinical problems, where treatment with interferons or isotretionin has been discussed as an alternative (27).

Immunoenhancing agents

Interferons alfa (leukocyte derived), beta (fibroblast derived) and gamma (lymphocyte derived), all being produced by recombinant DNA technology, would seem applicable for treatment of GPVI, inducing antiviral, antiproliferative and immunoenhancing effects (reviewed by Greene (115)). Topical as well as intralesional and parenteral administration has been tried with most varying results, responses usually being dose related; major limitations are high costs, influenzalike side-effects and questionable efficacy (115). Immunotherapy with inosine pranobex has also been tried, but without significant effect compared to that of placebo (67). Some authors have also recommended the use of topical idoxuridine cream (121, 122). Still, it would be desirable if an effective drug within this group will be developed in the future. The possibility to eradicate also the latent infections by stimulating the immune system would be a major advantage.

IMMUNOLOGY

Cellular immune response

Since antibodies do not penetrate the infected cell, they can not cause elimination of intracellular virus. If viral antigens are presented on the surface of infected cells, antibodies can help to lyse the cell. Viral antigens are presented for the T-helper cells by macrophages via major histocompatibility complex (MHC) class II molecules. In the epidermis this function is provided by the Langerhans cell (LC). These cells are reduced in number both in CIN and condylomas, which might contribute to the establishment of a persistent infection. (213, 331). Intraepidermal CD4 positive T-cells are also depleted in genital warts and in premalignant disorders (313). The cytotoxic CD8-positive T-cells, recognize viral antigens as short peptides presented on the surface of the cells in the context of MHC class I molecules. The presence of a specific cytotoxic T-cell response against papillomavirus-infected cells has not yet been demonstrated for humans, although the viral peptides with capacity to bind MHC class I have been identified (148).

It has long been known that patients with diseases that affect the cellular immunity, such as HIV-infected persons (132, 280), or patients with iatrogenic decrease of cellular immunity, such as renal transplant recipients receiving immunosuppressive drugs (15, 88, 94, 243), have severe problems with wart virus infections of both skin and genitalia, including preneoplastic and neoplastic lesions. Warts often disappear after elimination of immunosuppressive drugs. In contrast, hypogammaglobulinaemia patients do not have this problem (320). This observation suggests that the cellular, rather than the humoral immune response, is necessary for protection against clinical HPV infection (212). The capability of becoming sensitised to 2.4-dinitrochlorbenzene (DNCB) is used as an indicator of cell-mediated immunity. The incidence of DNCB contact sensitization has been reported lowered among a number of patients with skin warts, but not among patients with condylomata and Bowenoid papulosis (111, 225). In condylomata patients, no correlation has been found between DNCB sensitization scores, the number of warts and the response to treatment (338).

The potential relevance of cell-mediated immunological regression is elucidated by several clinical observations. Thus, concomitantly with spontaneous wart regression, the afflicted skin is often reddened and itching (24, 310), when biopsy samples generally show a dermal mononuclear cell infiltrate (311), and also the presence of epidermal inflammatory cells (140). In contrast, there is a low number of inflammatory cells in skin warts (329). Removal of one wart by therapeutical methods or trauma to a wart, often results in regression also of other warts, probably due to an immune response induced by release of antigens (284), another observation being in line with the suggestion that regression of warts is mediated by cellular immunity.

SEROLOGY

In the humoral immune response, antibodies are produced by plasma-cells. Effective triggering of an antibody response is

dependent on assistence from CD4+ T-helper cells. Unlike T-cells, antibodies can bind to any type of molecule (258).

Composite antigens in serology

Antibodies to wart virus in humans were first reported in 1965 with the demonstration of antibodies reacting with virus particles in electron microscopy (3). The early studies used crude wart extracts (227, 244-246, 332) or HPV 1 virions (151, 330) pooled from skin wart biopsies as antigenic targets. Antibodies were found to increase slowly during disease (64, 244). Follow-up studies of patients with warts, revealed that IgG represented a somewhat favourable prognostic sign for wart regression (245, 246). It is possible that these IgG titers seen at rejection have reflected a secondary event during cure, when wart cell destruction leads to exposure of PV antigens. Although individuals with the presence of antibodies can develop new warts, the antibodies may reduce the wart affected sites when reinfection occurs (334). All these early studies used composite antigens containing a multitude of epitopes, as antigen targets in the serological assays. In recent years several studies using viral fusion proteins expressed in bacteria, have been performed. The problem, when several epitopes are studied in the same assay, is to distinguish the response to useful epitopes with sufficient sensitivity and type-specificity from the ones lacking specificity. In a study performed by Jenison et al (143), using recombinant proteins encoded by E2, E7, L1 and L2 of HPV types 6, 16 and 18 in Western blot assays, no differences in the antibody responses were found between STD patients and children. In some studies there has, however, been significantly different prevalences of antibodies to HPV fusion proteins between cervical cancer patients and controls (145, 170). Antibodies to HPV 11 virions propagated under the renal capsule of nude mice, in enzyme-linked immunosorbent assay (ELISA), were found among condylomata patients (31), and were also found to decline among patients cured from condylomata compared to patients with recalcitrant warts (32).

Synthetic peptides in serology

There are several advantages with the use of ELISAs based on synthetic peptides. Sensitivity can be increased by an increased antigenic density. Reliability is gained by the use of a single, chemically defined epitope and the technique is simple and has a low cost (77).

There are differences between different epitopes in their rate of induction of antibodies after infection, with the response against some epitopes developing rapidly after infection (paper VI) and others only decades after infection when HPV-positive tumours occur (217). The immunological memory may also be different for responses against different epitopes, in that some may disappear quickly after clearance of infection, while others persist longer (179).

The major reactive epitopes are depicted in Figure 2 (modified from Dillner (77)). E6 has two epitopes; E6:4 (81) and E6:10, both highly reactive, but the former only moderately disease associated (77). In E7 there are two extensively studied epitopes, the E701 and the E7:5. Both are associated with cervical cancer (76, 81, 84, 165, 217, 301), and IgA against E7:5 is also found in the cervical secretions of condyloma patients (83). In the aminoterminal end of E7 there is also a moderately disease-associated epitope, E7:1 (76, 165, 301). In the middle of E1 there are two overlapping cervical cancer-associated epitopes (82). Two well defined epitopes are present in E2: E2:9 and 245. Antibodies to the former are present in the sera of patients with cervical and anal cancer (84, 124). The peptide antigen 245 has been extensively characterised; the antibody response against this epitope has been found to be associated with CIN (78, 180, 249, 299), and cervical cancer (81, 176, 177, 194, 249). A decline in titers has been found after treatment for CIN (91, 180) and after treatment for cervical cancer (only for IgA) (176). An association with cervical cancer has also been found for the antibody response against the corresponding peptide from HPV 18 (245:18), in particular with adenocarcinoma (81, 178). Three weakly disease-associated epitopes are present in E4 (76, 145, 300). In the middle of L2 there are two closely related epitopes, however not cross-reactive. The L2:49 peptide is associated with

Major defined epitopes in HPV

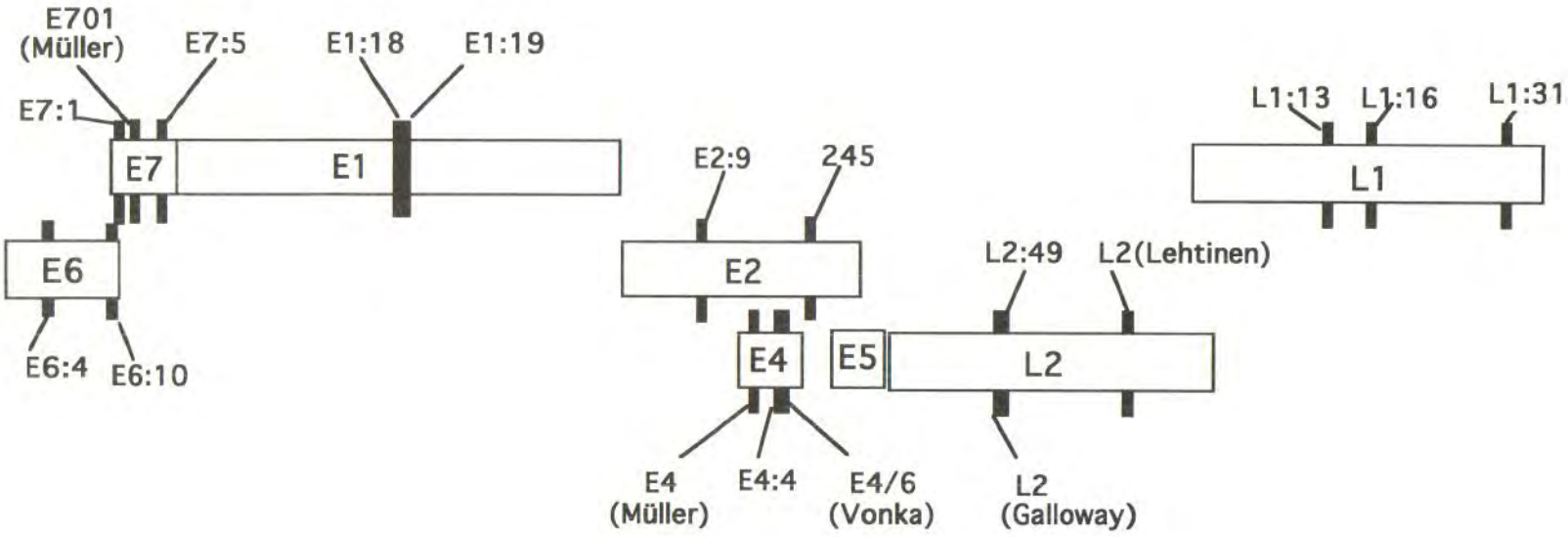

Fig. 2

previous condylomas (paper V), while the L2 (Galloway) epitope is not disease associated (109, 142). In the L2 carboxyterminus, Lehtinen et al have reported an HPV type-restricted epitope to which IgA is preferentially found among CIN patients, and where the titers decline after spontaneous regression of the lesion (179). In the middle of the L1 region, there is a well characterised epitope (L1:13), to which an increased antibody response is seen in patients with cervical cancer (79, 81). The L1:31 epitope is located in the carboxyterminal. An increase in the IgG response for the HPV 6 peptide has been seen among patients with previous condylomata (paper V).

HPV virus-like particles

HPV 16 L1 plus L2 virus-like particles (VLPs) have been assembled via recombinant baculoviruses-mediated expression in insect cells (155). In ELISA assays, sera of 59% of women PCR positive for HPV 16, tested positive compared to 6% of DNA negative women and 9% of women positive for HPV DNA 6/11 (154). Seroconversions against HPV 16 VLPs are seen concomitantly with HPV 16 DNA acquisition (paper VI) and among teenage women who acquire HPV 16 infection a majority will seroconvert (4). Similar particles of other HPV types have also been produced (154, 333), although their serological reactivity has only recently been characterised (paper VII).

EPIDEMIOLOGY

The classical genital warts, have an incidence rate of about 1% among sexually active people, with a peak incidence in the age groups 19-22 years and 22-26 years for females and males, respectively (55, 229). Most infections will, however, disappear spontaneously without causing clinical disease (326). Condylomata are mainly transmitted sexually, although the existence of non-sexual transmission has been discussed (272).

When analysing epidemiological studies, it is important to relate the findings with the HPV DNA hybridisation method used, since they

may differ regarding sensitivity, specificity and interassay reproducibility (73). The results are also dependent on the choice of control group in relation to cases, since HPV positivity is dependent on both age distribution, geographic origin and social class. The lack of differences between different patient groups in some studies may also be due to misclassification of patients in regard to HPV infection status; even minimal misclassification on epidemiological findings may significantly alter the results of the study (106).

Genitoanal HPV infection in children

HPV can infect the genitoanal area of a child in several ways: from the mother before delivery, during delivery, by contact with the genitoanal area occurring in non-abusive situations through autoinoculation via finger warts on the child´s own hands and through sexual abuse (231). The role of sexual abuse among children with genital HPV infections has been intensively discussed lately and the estimations of the importance of this route of transmission vary considerably between different studies. In a review of the literature, Gutman et al (119) found that the reported proportion of children with genital warts where an etiology of sexual abuse could be documented varied between 4-91%.

Contagion with HPV 6 and 11 via an infected birth canal can cause recurrent respiratory papillomatosis in the infant (285). The clinical triad of a firstborn to a teenage mother and vaginal delivery is typical among children with juvenile onset of respiratory papillomatosis (147). The condition is rare among children delivered by Cesarian section (285). HPV DNA presence has also been reported in healthy newborns; HPV DNA was found in 33% of nasopharyngeal aspirates of newborns (282). A few cases of HPV DNA positivity in foreskins of infants undergoing routine circumcision has been reported (259). Recent investigations have not been able to confirm the findings of HPV DNA in newborns; using the highly sensitive PCR technique, Chen et al (52) did not find any HPV DNA on the genital mucosa of 98 neonates.

The incubation time of warts in children is uncertain, but laryngeal papillomas usually present between two and three years of age

(105), although the condition may be presented up to the age of 12 years (171). In a survey of results from different studies, Lacey (171) reported that 75% of genital warts in children are caused by HPV 6 or 11 and only 13% by HPV 2, indicating that transmission of hand warts to children with resulting genital warts is uncommon.

Men

Most epidemiological studies have been performed on women in younger age groups. A few studies have been conducted on men, and these usually on patients attending STD clinics, a risk group for genital HPV infection. In a Swedish study, nearly one-third of patients attending an STD clinic had HPV-associated lesions (296). In a study of healthy men (non STD patients) analysing penile smears, 5.8% were HPV positive (117). In a most detailed study on Finnish conscripts (130), classical genital warts were present in 5.6% of the young men, while acetowhite lesions were found in as much as 35.0%, of which 14.1% exhibited a typical penoscopical pattern. In PAP smears, koilocytosis were present in 6.5% and 16.5% harboured HPV DNA using the PCR assay. In a similar study on Swedish army conscripts, 12% of men (without acetowhite penile lesions) were HPV positive with PCR (150). The natural course of HPV infection in men was studied by van Doornum et al (326) who enrolled 48 men reporting >5 heterosexual partners during the last six months into a study. Serum samples and brush samples were taken from multiple genitoanal locations at multiple occasions with 4-monthly intervals. Totally, 31% of the men were HPV positive at enrolment using PCR, with HPV 16 and 18 as the most common HPV types. Another 29% of the men acquired HPV infection during follow-up. Most of the infections were transient and disappeared from one visit until the next: only 6% of the infections were detected at two consecutive visits. Similarly, only 8% of infected males had colposcopically detectable lesions. Most of the infections were found on the penis (65%) and the rest (35%) were found in the anorectal region. No men carried HPV DNA in the oral mucosa.

Women

The HPV prevalence is very much dependent on age. This age dependency has been elegantly shown in a large Dutch population-based study of four different age groups of women, all exhibiting normal smears. With the PCR method, the maximum prevalence was found in the age group 20-24 years, with a rate of 20-25% HPV DNA positivity. On the other hand, after the age of 35, a constant level of 1-2% HPV 16/18 was observed (207). Several other studies have also reported high prevalence rates of HPV infection in young women (195, 323, 353). The prevalence rate is directly correlated to the number of sexual partners (262); the number of sexual partners appears to be the only independent risk factor for HPV infection (95). In one study, 21% of women reporting only one partner were PCR positive for HPV compared to 69% of women with 10 or more partners (182). An association with a high number of partners and prevalence rates of HPV infection, has also been found in other studies (104, 214). However, the number of recent sexual partners (during the last year) seems to be a stronger predictor for HPV positivity than lifetime number of sexual partners (41). The natural course of HPV infection was described in longitudinal studies of adolescent women who were followed for 13 months; 29/51 (57%) of women were HPV-positive on one occasion, but only 4/51 (8%) women were positive on both tests. Of the HPV DNA positive cases, only 1/4 was infected with the same HPV type at both tests (261). In a Swedish study, Evander et al (95) reported an HPV prevalence of 21% among young women. When retested after two years, only 2/276 women had persistent infection with the same HPV type. Taken together, these longitudinal studies indicate that the natural history of HPV infection is a rapid spontaneous clearance, occurring among the vast majority of infected persons. Only if the infection persists can it induce HPV-associated lesions, such as CIN.

A low prevalence or absence of cervicovaginal HPV in virginal women supports the finding that the infection is primarily sexually transmitted. In two studies of vulval samples of virginal women, some cases of HPV infection were found by histological appearance or by PCR (182, 312). However, as extravirginal tissue was sampled, vulval infection could have occurred by non-penetrative sexual

activities. This is supported by the observation that HPV DNA was found in 3/15 vulval swabs but in none of the cervical scrapes from 15 virginal women (17). In two other studies, all virginal women were negative for HPV DNA with the PCR assay (97, 263). Early studies of HPV seroprevalence in persons with no sexual experience were unconclusive (143, 145) because non-characterised serological methods were used. In a recent Swedish study, virginal women were both negative for HPV DNA by PCR and sero-negative for antibodies against HPV 16 capsids (4), a serological method which has a documented specificity for HPV infection (154). Among women with new HPV 16 infection, antibodies to HPV 16 capsids are induced concomitantly with infection (paper VI).

Partner studies

Male partners of women with cervical lesions have penile lesions in about 50-70% of cases (16, 43, 46, 131, 169, 196, 273). Interestingly, the type of lesion found correlates within the couples. Barrasso et al (16) found that condylomata were present in 41% of partners to women with condylomata, but in only 5% of women with CIN. Penile intraepithelial neoplasia was found in 32% of partners to women with CIN, but in only 1% of women with benign flat condylomas. Monsonego et al (211) found that 220/410 (54%) of male partners to women with GPVI had penoscopic lesions. However in only 32% of cases did both partners carry the same HPV type. Ho et al (133) studied sequence variants of HPV 16 from couples who were both infected. Only in 4/8 couples where both partners had HPV 16 infection, was an identical HPV 16 variant found. Thus, the finding that both partners in a couple are infected with HPV 16 does not prove that both persons are infected with the same virus strain. The reason for the low concordance of viral type between partners is not clear, but could be a reflection of the fact that both clearance of infection and acquisition of new infection are common events.

Among steady couples with established condylomatous lesions, treatment of one partner does not affect the treatment results in the other (166, 167). In present clinical policy, condom use is no

longer recommended in steady couples where the man and the woman both receive treatment for GPVI.

CIN AND CERVICAL CANCER

Cervical cancer, the second most common female cancer worldwide (241), has long been regarded as a sexually transmitted disease (322). Already in 1842 (254) it was reported that cervical cancer is extremely uncommon among women with no sexual experience, but it would take more than a century to find the etiology and to more systematically define the risk factors.

Several risk factors for cervical cancer related to sexual behaviour have been identified, such as age of first intercourse and number of lifetime partners (36), i.e. the same risk factors as for genital HPV infection. The "epidemiologic profile" of CIN patients is that these have more sexual partners, more cigarette smoking, earlier ages at first sexual intercourse and lower socioeconomic status (270). These epidemiological correlates for CIN were fully explained by HPV infection (270). Parity for multiple children is a weak to moderate risk factor for invasive disease in high-incidence areas for cervical cancer (156, 283) and HPV infection can not explain the risk associated with parity (270). It is speculated that sex hormones during pregnancy exert immunosuppressive effects (157). The male behavior also plays a role in the cervical carcinogenesis. Among women with only one lifetime partner, other sexual relationships of the husbands both before and during marriage, increased the wives´ risk of having cervical cancer by 6.9 times (2).

For more than a decade, HPV has been suggested to play an important role in carcinogenesis (356). Viral genomes of the "high risk group" have been found in up to 100% of cancer samples, the detection rate being dependent on the hybridisation method used (8, 69, 247, 324). An increasing total HPV prevalence has been noted with increasing severity of dysplasia from 71% in mild dysplasia to 100% in carcinoma in situ samples using PCR (69). In a study from Colombia and Spain (33), the relative risk (RR) for cervical cancer for HPV positive persons was estimated to be 24. In

an American study, Lorincz et al (186) estimated the RR for persons with an oncogenic HPV type to have a high-grade lesion to 65-236 (depending on HPV type) and 31-296 for invasive cancer.

The role of other sexual transmitted agents apart from HPV is under intense investigation. Antibodies to chlamydia trachomatis were found to be an independent risk factor in a case-control study for CIN III and cervical cancer (70) and also in prospective seroepidemiological studies (175). Cytomegalovirus has also been suggested as a cofactor (286).

Although there never was any conclusive identification of herpes simplex virus (HSV) DNA in cancerous tissue, the theory of HSV as a causative agent of cervical cancer was widely propagated in the 70ies (208). While some initial serological case-control studies found an association between HSV and cervical cancer, several subsequent large prospective studies found no association (176, 346). During the last decade, the support for HSV as an important factor for cancer development, has become weaker, and the virus now only remains useful as a marker of sexual activity (172).

The role of oral contraceptives in the development of cervical cancer, has been extensively studied. The results are conflicting, since some studies support an association (38, 220) and others do not (238, 293). A protective effect has been ascribed to the use of barrier contraceptives and spermicidals (47). Several studies indicate that cigarette smoking may increase the risk of cervical cancer development (57, 146, 174, 184). As shown by Hellberg et al (126), nicotine and cotinine accumulate in the cervical mucus in measurable quantities, providing an explanatory model for how smoking could be a risk factor or risk modifier, and not merely a confounder due to differences in the sexual behaviour between smokers and non-smokers. However, most of the recent epidemiological data indicate that smoking is merely an indirect association, reflecting differences in HPV exposure between smokers and non-smokers (270). The role of diet has also been discussed; a high consumption of fruits and vegetables containing beta-carotene, vitamin C and folate has been reported to be protective for cancer development (128, 292). Genetic factors

important for cancer development have recently been studied: associations between certain HLA DR-DQ alleles and cervical cancer have been reported (7).

Natural history of CIN

In a prospective study (163) 241 cytologically normal, young STD patients were followed with colposcopy and dot blot DNA tests every four months for a mean of 25 months. Of these, 28 women developed CIN II or III, suggesting that development of high grade CIN is a comparatively frequent and rapid effect of persistent HPV infection. Interestingly, in only 10/28 cases was CIN I noted before CIN II or III was observed. The risk was highest for women positive for HPV 16 and 18.

Already in 1952 it was found that carcinoma in situ (CIS) could regress spontaneously (267). Koss et al (161) reported that taking a biopsy of the cervix could alter the natural course of the disease, leading to cure also if the lesion was beyond the margins of the biopsy. To circumvent the possibility of biopsy influencing the course, Richart & Barron (253) prospectively followed 557 women with cytologic abnormalities, and did not take any biopsy before CIS was suspected. They noted a progression rate to CIS of 160 per 1000 cases per year. These women represented, however, cases with persistently abnormal smears, since three consecutive abnormal smears were required for inclusion into the study.

In a Swedish study on 555 women followed for a mean of 39 months, 62% of mild dysplasias regressed, whereas 16% progressed to high-grade dysplasia or cancer in situ and 22% persisted (219). The authors calculated a risk of progression of 250-800 per 100.000 cases per year, a figure considerably lower than reported by Richart & Barron. Evans and Monaghan (96) have demonstrated a progression rate of 16% of flat cervical condylomata to CIN II/III within one year. In the large prospective Kuopio study, Kataja et al (149), reported that 58% of cervical HPV infections regressed spontaneously, 15% progressed and 25% persisted; CIN II, CIN III and HPV type 16 being the most significant independent prognostic factors for clinical progression. In this study, biopsies were taken

during the follow-up period, which could have influenced the natural course.

VULVAR CANCER

As the vulva and cervix are usually exposed to the same external influences, such as HPV infection, the question has been raised whether HPV is important for the development of vulvar cancer as well. In the literature, HPV DNA has been found in 0-80% (overall 52%) of vulvar carcinomas (reviewed by Ansink (5)). A past history of genital warts and cigarette smoking has been noted as risk factors (37). Vulvar carcinomas can be subdivided into three forms; Keratinizining squamous cell carcinoma (SCC), warty carcinoma and basaloid carcinoma (136). The first type is the most common, occurring mostly in elderly women and is rarely associated with adjacent VIN III. In contrast, warty and basaloid carcinoma patients are younger, dysplasia is found in 78% in adjacent areas and the lesions are HPV positive in 75% of cases (136).

BOWENOID PAPULOSIS AND PENILE CANCER

Bowenoid papulosis was in 1970 described by Lloyd (185) as a multicentric Bowen´s disease in both sexes, characterised by multifocal genital lesions with a histology similar to that of CIS, although the clinical course is benign with a high degree of spontaneous regression (135, 152, 290). HPV type 16 is usually found in the lesions (116, 137). Originally described as papular, the lesions can also be macular ((16); paper II, III). Although HPV genomes, mainly of the oncogenic types, have been found in penile cancer (191, 201, 260) the role of the infection in the etiology is not understood (129). Much lower rates of HPV DNA detection have been reported in penile cancer compared to severe penile intraepithelial neoplasia (129). In a case-control study by Maden et al (191), risk factors for penile cancer regarding personal characteristics and habits were analysed. The risk was increased for men who currently smoked (2.8 times), men with a history of condylomata (5.9 times) and men who were never circumcised (3.2

times). Epidemiological evidence for lack of neonatal circumcision as a risk factor for penile cancer has also been reported earlier (127, 275), suggesting that chronic irritation is an important risk factor (191). The incidence of penile cancer in Scandinavia and Japan with an uncircumcised male population with good hygienic standard is one-tenth than that in developing countries (276). There is also an excess of deaths from cervical cancer among wives to males with penile cancer (125, 197) also suggesting a sexual transmission.

AIN AND ANAL CANCER

An increase in the incidence of anal cancer during the past 30 years has been reported (108, 112). The tumour is more common among women and has a peak incidence between 60 and 70 years of age (113). In the United States, a rapid increase among men in the San Francisco Bay area, especially among never-married men (a marker for homosexuality) compared to ever-married men has taken place (206). An association between AIN and a history of anal warts, frequency of anal intercourse, presence of HIV infection and immune dysfunction with a low CD4/CD8 ratio, has been reported (reviewed by Wells (351)). In a recent study (205), AIDS patients were found to have a RR for anal cancer of 84 among homosexual patients and of 38 among non-homosexual patients. The RR of anal cancer was also increased among HIV-positive subjects up to five years before AIDS diagnosis. Homosexual men with advanced HIV disease develop anal cytologic abnormalities over quite a short period of time and should therefore be followed with anoscopy and cytology (233). HPV 16 DNA has been found in AIN (228, 277) as well as in anal SCC (234). As many as 55% of anal cancer patients have serological signs of HPV 16 infection (seropositivity against HPV 16 capsids) compared to only 4% of matched healthy controls (123).

Interestingly, it was recently published that a histologic diagnosis of anal HPV infection or AIN was found in 48% of women with invasive vulvar cancer, suggesting an etiologic relationship between genital and anal squamous neoplasia (228). Furthermore, an

increased incidence of AIN has been reported among women with high grade CIN (277).

ORAL INFECTION

Apart from the normal oral mucosa, HPV DNA has also been found in some oropharyngeal SCCs (134, 288, 308), but it remains unclear whether the HPV detected in these tumours is causally associated with tumour development or merely represents a passenger infection (107). HPV DNA has also been found also in various benign oral lesions, including squamous cell papillomas and focal epithelial hyperplasia (reviewed by Chang et al (48)). HPV types 16 and 18, as well as HPV 11 have been detected in some oral SSCs, and HPV 2 also in some carcinomas of the tongue and in oral verrucous carcinomas (247). It is unclear how common oral HPV infection is in the general population. E.g. Jalal et al (141) found HPV DNA in the oral mucosa of 44% of healthy adults, whereas van Doornum et al (326) who enrolled a cohort of 158 subjects with >5 sexual partners during the last six months and tested them for HPV at multiple occasions at 4-monthly intervals found that only 1/158 subjects had an oral infection, and only at one of the test occasions.

HPV IN SKIN CANCERS

In the rare hereditary skin disorder epidermodysplasia verruciformis, HPV DNA has been detected in the skin lesions. Clinically the patients present with red, flat, wartlike lesions that progress to SCC in 30-50% of cases (247). HPV types 5 and 8 are the most common HPV types associated with these tumours, which develop on sunlight-exposed skin areas, indicating that UV-irradiation is an important co-factor (139).

Renal transplant recipients have an increased risk of viral warts as well as cutaneous SCCs (15, 93). Various HPV types have been found in a proportion of both the benign and malignant lesions, including the EV-associated HPV 5 (247). The incidence of SSCs in

sun-exposed skin of renal transplant patients is 21 times that of the normal population (237).

The distal digit and the periungual skin represents the most frequent anatomical location where HPV DNA is found in oncogenic types and SCCs at non-genital sites (216), suggesting a transmission through genital-digit contact (92). Reviewing the literature, Sau et al (264) reported that the presence of HPV 16 DNA in periungual Bowen´s disease and SCC was confirmed by at least five studies. In contrast, histologically benign lesions in the same location only contain the benign HPV types (224). However, most HPV-associated digital SCCs represent low-grade malignancies in terms of metastatic potential; in situ lesions can usually be treated with cryosurgery or laser vaporization, and cone excision for minimally invasive lesions, with the exception of periungual and subungual carcinomas that are usually more thickened (215). Also on other non-genital sites, the presence of HPV DNA in SCCs among immunocompetent individuals has been reported albeit in a low proportion of cases; if HPV plays a role in the carcinogenesis of these cases is not clear (247). The occurrence of HPV DNA in skin tumours apart from SCCs has also been studied; HPV types 9, 16, 19, 25, 37 have been found in some keratoacanthomas (247).

HPV IN OTHER CANCERS

Involvement of HPV in the pathogenesis of esophageal cancer was first suggested by Syrjänen, who reported that these tumours frequently contained histological changes similar to those induced by HPV infection (303). Several reports have also detected HPV DNA in esophageal tumours (49, 321). Interestingly, an increased frequency of HPV 16 capsid seropositivity in esophageal cancer cases has recently been detected (80).

Laryngeal papillomas can develop in children due to infectious virus from the mother. Malignant conversion of laryngeal papillomas can occur after x-irradiation of the papillomas (192, 248). Brandsma & Abramson (34) reported that 14% of patients receiving x-irradiation developed carcinomas. The predominant

part of solitary adult papillomas represent a premalignant condition, with smoking as a known co-factor promoting malignant transformation (50). HPV DNA types 6, 11 and 16 have been demonstrated in some laryngeal carcinomas (307). HPV DNA has also been found in rare cases of bronchial carcinomas (304), and lung carcinomas (298).

Nasal papilloma was described by Ringertz already in 1938 (255). Both HPV 11 and 16 DNA have been detected in the tumours of patients with papillomas some of which later undergo malignant transformation (305). Regarding ocular pathology, HPV DNA has also been found in malignant lesions of the conjunctiva and cornea (203).

A few reports have found HPV in urinary bladder cancers. In one study (6), HPV DNA was found in 81% of carcinomas and in 33% of normal bladder specimens using the PCR assay. Another study reported HPV DNA in 10% of transitional cell and in 17% of papillary carcinomas (39), whereas other studies only detected HPV DNA in 1-2% of bladder carcinomas (53, 193).

A Norwegian follow-up study on 37.000 women with a history of cervical CIS found an increased risk for development of malignancies of the vulva/vagina, nose/nasal cavities, respiratory tract including lungs, skin (excluding melanomas and basaliomas), oesophagus, tonsils and the urinary bladder (28). The over-representation of these cancers among former CIS patients suggests the existence of a shared risk factor(s) for these cancers. Smoking habits and HPV infections are the most likely candidates for such shared risk factors.

Aims of the study

To evaluate the efficacy of available treatment modalities for penile and anal warts (I, IV).

To compare the success of anal wart treatment in heterosexual and homosexual men (IV).

To define specific diagnostic criteria for subclinical penile HPV infection and to compare diagnostic methods including penoscopy, histopathology and HPV DNA hybridisation assays (II).

To elucidate the diversity in the clinical presentation of GPVI (III).

To study the development of the HPV antibody response among patients who acquire HPV infection (VI, VII).

To study the development of HPV antibodies during the course of condylomatous disease as well as among patients who have become cured of condylomata (V, VII).

MATERIALS AND METHODS

Paper I. A total of 230 men who presented during a year to the sexually transmitted disease (STD) clinic at the South Hospital, Stockholm due to genitoanal warts mostly afflicting the penis, were included into a retrospective evaluation of patient medical charts. Of these, 72 (31%) had been referred to the clinic due to failure of previous therapy. Patients were interviewed about sexual preference, warts were counted and depicted on a standardized chart being used for each visit when the warts were monitored and stratified according to distribution of lesions. In cases of anal warts, proctoscopy was performed. Perianal warts refer to warts occurring ⩾1cm laterally of the anal verge and the term intraanal warts is used when warts occur in the anal canal and are not visible without the aid of a proctoscope. The urinary meatus was examined with digital meatal eversion. Treatment for penile warts included chemical therapy with podophyllotoxin, or surgical therapy with scissor excision and/or diathermy, while all anal warts were treated surgically. Patients were regularly checked up 2-3 months after therapy until cure.

Paper II. A series of 91 men attending the STD clinic at the Karolinska Hospital, Stockholm due to penile symptoms or as partners to females with known GPVI, were included. Penile examination included 5% acetic acid testing combined with colposcopic magnification "penoscopy". Biopsies were taken from acetowhite lesions classified as being either typical (well demarcated lesions with slightly elevated borders and the occurrence of punctuated capillaries), conspicuous (features as for typical lesions except for the presence of punctuated capillaries) or as non-typical (exhibiting ragged borders and lacking punctuated capillaries). Half of the biopsy specimen was used for routine histology (hematoxylin/eosin staining) and in situ hybridisation using biotinylated probes for HPV types 6, 11, 16, 18, 31, 33 and 42 (306). Lesions were histologically classified as typical (the presence of epidermal hyperplasia, acanthosis, hyper- and parakeratosis and koilocytosis), conspicuous (all of the criteria above except for

koilocytosis), and any degree of intraepithelial neoplasia was recorded. Non typical histopathology included an eczematoid reaction only, normal skin or other dermatological conditions. The other half of the biopsy specimen was analysed with Southern blot using the cloned genomes of the HPV types 6, 11, 16, 18, 31 and 42 as probes and with PCR using type specific primers for HPV 6 and 16 as detailed in the paper.

Paper III. Twentythree symptomatic males attending the STD clinics at the Karolinska Hospital and at the South Hospital, Stockholm during a four-year period, were included. All men suffered from long-lasting and/or recurrent penile itching, burning, tenderness, dyspareunia, oedema and/or inguinal pain. They all presented with clinical signs of balanoposthitis including erythema, and in some cases the occurrence of fissures, oedema, and/or inguinal adenopathy as well. They were prescribed topical anti-inflammatory therapy with Daktacort^{R} cream (miconazol and hydrocortison) twice daily for two weeks, according to current clinical routine. Patients who still exhibited acetowhite lesions after local therapy with typical or conspicuous acetowhite penoscopical patterns (as detailed in paper II), were included. Biopsies were taken for light microscopy evaluation using the criteria encountered for in paper II, in situ hybridisation (using biotinylated full-genomic probes for the HPV types 6/11, 16/18, 31/33 and 42 (306)) and for PCR (using L1 consensus primers according to Manos et al (195)) (the same biopsy specimen was used for all three analyses).

Paper IV. The medical charts of 111 men who attended to the STD clinic at the South Hospital, Stockholm for therapy primarily of anal warts during a 19 months period, were retrospectively analysed. Samples were taken for other STDs as in study I. Warts were classified as acuminate, papular or flat, counted and depicted on a special evaluation chart. Biopsies were taken for histopathology (hematoxylin/eosin staining) and in situ HPV DNA analysis using ^{35}S-labeled full-genomic probes for HPV types 6, 11, 16, 18, 31 and 33 (306). Of the 111 men enrolled for biopsy sampling, 81 were included in the therapy protocol; of these 76 were treated at

the STD clinic. Treatment included chemical or surgical therapy as in study I. The men were followed up until cure.

Paper V. Serum samples were collected from 1) asymptomatic men with no history of genital warts, who served as controls (n=116); 2) men with current condylomas (n=22) and 3) men who had previously had condylomas (n=21), attending the STD clinic at the Karolinska Hospital, Stockholm, Sweden. Serum samples were also collected at the STD clinic at the Huddinge Hospital, Stockholm; 90, 25 and 12 men belonging to groups 1, 2 and 3, respectively. The patients visited the clinics for other reasons than condylomas, and were not acetic acid tested. Serum samples were analysed with ELISA using synthetic peptides from the E2, L1 and/or L2 regions for HPV types 1, 8, 6, 11, 16, 18, 31 and 33, as previously described (76, 79).

Paper VI. Between 1988 and 1990 the participants of the study had visited the STD Clinic of the Municipal Health Service of Amsterdam. The patients were all heterosexual, 18 years of age or older and had had sexual contact with at least five partners during the last six moths before entry, and were willing to visit the clinic every four months. Of the 354 women and 257 men who entered the study, 110 women and 48 men could be followed (for further description of the cohort, see van Doornum et al (326)). At each visit, brush samples were taken for HPV DNA detection with PCR using type specific primers for HPV 6/11, 16, 18 and 33. Samples from the males were taken from the mouth, anus, rectum, coronal sulcus and urethra. From females, specimens were analogously taken from the mouth, anus, rectum, cervix and from the labia minora. Serum samples were taken at each visit and analysed for eight HPV-derived synthetic peptides: from the E2, L1 and L2 regions (five HPV 16-derived, one HPV 18-derived and one HPV 6-derived peptide(s)) as well as against denatured BPV. Initially, only the first and last serum sample from each patient were analysed. Altogether 16 patients were found who initially were seronegative and later became seropositive, and the sera from these patients were furher analysed. These patients, as well as a group of individuals (n=22) who at the first visit were HPV DNA negative

and later became HPV DNA positive, were also analysed for HPV 16 capsids in ELISA.

Paper VII. Twentysix patients were treated for condylomata at the STD clinic at the Karolinska Hospital. At each visit, serum samples were collected and analysed for five HPV-derived synthetic peptides from the L1 and L2 regions (four HPV 6-derived and one HPV 16-derived peptide(s)), as well as for HPV capsids of types 6, 11 and 16. From the Dutch cohort described in study VI, we also selected 17 patients for study, who at the initial visit were PCR negative for all HPV types tested, but who later became HPV 6 positive.

RESULTS

Therapeutic outcome in patients treated for penile and anal warts

In order to evaluate the efficacy of available treatment modalities for condylomas in men, two retrospective follow-up evaluations, based on review of standardized patient files, were performed on 230 men afflicted predominantly with penile warts (I) and of another 81 men attending mainly due to the presence of anal warts (IV).

In study I 75% of the men presented with condylomas in the preputial cavity and 17% with anal warts. Coexistent penile and anal condylomas occurred in 12/23 hetero- compared to in 1/15 homosexual males ($p<0.05$), while intra-anal warts were most common in the homosexual population (13/15 versus 8/23) ($p<0.001$). Of the 230 men 28 (12%) required referral to other hospital units, 19 men to the proctology unit due to massive anal growths and another nine patients to the urology department due to warts in the distal part of the urethra. Of the remaining 202 patients being treated in the STD department, 156 (77%) were cured within one year, while 46 (23%) exhibited recurrences and/or re-occurrences over a longer time period. Altogether 77 (49%) of the 156 men were cured already after a single treatment and 128 (82%) men became wart free after 1-4 sessions. The remaining 28 (18%) men required numerous therapy sessions being in the range of 5-12.

Of the 81 men in study IV, 76 (94%) were treated at the clinic, while five (6%) required a direct referral to the proctological unit due to bulky anal warts. At some time during the study period, 14/76 (18%) men required assistance from the proctology unit and a single case (1%) from the urology department. Also in this study concurrent anal and penile warts were significantly most common in the hetero- (23/25 during the study period) compared to the homosexual group (4/73) ($p<0.001$). Perianal warts were more common in the heterosexual group (16/38 at inclusion versus 16/73 homosexuals) ($p<0.05$), while intraanal warts were more common among the homosexual men (33/73 versus 1/38

heterosexuals at inclusion) ($p<0.001$). Acuminate condylomas predominated among the biopsied warts, representing 64% (n=87); 24% (n=33) and 12% (n=16) papular and flat lesions, respectively. Anal intraepithelial neoplasia was present in 28% (n=38) of anal biopsies, exhibiting grade I and II in 27% (n=35) and 2% (n=3), respectively. No case of AIN grade III was deteced. Using ISH, applying specific probes for the HPV types 6, 11, 16, 18, 31 and 33, altogether 87% (109/125) of biopsies were HPV DNA positive. The HPV types 6 and 11 predominated, being present in 102 (94%) of the 109 ISH positive cases. Another seven (6%) exhibited the HPV types 16, 18, 31 or 33. Regarding therapeutic outcome, it appeared that heterosexual men required a significantly higher number of treatment sessions than the homosexual men (mean 3.9 sessions versus mean 1.9 sessions) ($p<0.001$), and also needed a longer time of management until complete cure was accomplished (mean 13.1 months versus mean 5.5 months) ($p<0.001$). No difference in therapeutic efficacy was observed between HIV seropositive versus HIV negative homosexual men.

Diagnostic criteria for subclinical genital papillomavirus infection

For the purpose of determining penoscopic criteria for the diagnosis of subclinical GPVI, biopsies from various categories of acetowhite penile lesions were analysed with conventional histolopathology, as well as with different HPV DNA hybridisation assays including SB, ISH and the PCR (II). Penoscopic as well as histopathological criteria were stipulated in 91 men as being either typical, conspicuous or non-typical for GPVI. The predominant majority of biopsies were collected from lesions classified as typical/conspicuous by penoscopy (78/91; 86%) or by histology (66/91; 73%). HPV DNA was detected in 65/91 (71%) of the samples by at least one of the hybridisation assays.

Of the 78 biopsies collected from lesions classified as typical/conspicuous by penoscopic criteria, 56 (72%) showed histopathological signs being concordant with HPV influence, corresponding to a sensitivity of 85% and a specificity of 12% for these penoscopy criteria of GPVI as compared to light microscopy evaluation. Of 78 biopsies classified as typical/conspicuous by

penoscopy 55 (70%) were HPV DNA positive, correlating to a sensitivity of 85% and a specificity of 12% for the penoscopy criteria as compared with positive hybridisation results. None of the HPV DNA assays were found to increase the diagnostic potential for identifying GPVI lesions compared with conventional histopathology. Some degree of penile intraepithelial neoplasia was detected in 18 (20%) of the 91 biopsies, with PIN grade I, II and III being present in three, ten and five cases, respectively. Three samples showed the existence of other dermatological conditions (lichen sclerosus et atrophicus, verruca plana and balanitis circinata parakeratotica in one case each), while in five cases only normal epithelium was present.

Acetowhite symptomatic penile HPV lesions

In order to elucidate the diversity in clinical presentation of GPVI, we examined retrospectively biopsy specimens from either penoscopically typical or conspicuous acetowhite lesions of 23 men with various long-lasting penile symptoms, including burning (52%), painful fissuring (43%), discomfort during intercourse (39%), tenderness (26%) and itching (22%) (III). All men exhibited clinical signs of balanoposthitis. One man presented with penile oedema and another patient with a painful inguinal adenopathy. Biopsy samples from all of the 23 men exhibited light microscopical typical/conspicuous signs of HPV infection. Dermal inflammation was present in 17 (74%) of the biopsies, graded as mild, moderate and severe in seven, eight and two cases, respectively. As much as 17 (74%) samples revealed a picture of PIN, graded as I, II and III in six, seven and four cases, respectively. The ISH assay revealed the presence of HPV DNA in 13 (56%) of the biopsies; all of the analysed HPV types (6/11, 16/18, 31/33 and 42) appeared to be represented.

Antibodies among patients with previous condylomata

We studied the antibody response among 47 men with condylomas, among 33 men with a history of condylomas, as well as among 206 controls, against defined epitopes in the capsid proteins of HPV 6 (V). We found an elevation of IgG antibody responses among

patients who earlier had been afflicted with genital warts against epitopes in the L1 and L2 capsid proteins of HPV 6. The differences were significant, and the same result was found in two parallel series of patients enrolled at two different hospitals. No major differences were found between patients with visible condylomata and controls.

Antibodies among patients with HPV seroconversions and new infections

In order to determine when antibodies to HPV are induced in the course of infection, we studied a cohort of 158 patients attending an STD clinic, reporting sexual contact with five or more heterosexual partners during the last six months (VI). The patients were followed for a mean of 500 days, during which time they made 2-7 visits with a mean interval of 190 days between the visits. At each visit brush samples were collected from multiple genitoanal locations as well as from the oral mucosa for HPV DNA detection by PCR. Serum samples were also collected and used for the analysis of serological reactivity to synthetic peptides derived from the E2, L1 and L2 regions of the viral genome. Sera were also tested against disrupted BPV and intact HPV 16 capsids. Among the 158 patients, we found 16 patients who were serologically negative at the first visit and became positive during follow-up, denominated as "seroconverters". For five patients new HPV DNA was detected at the same visit as peak of antibodies, for three patients new HPV DNA was found at the visit before the antibody peak and for one patient HPV DNA was found at the visit after the peak. In general, antibody peaks for several different HPV-derived antigens, were induced at the same visit, indicating that the antibody responses were induced by the same infection.

The kinetics of the antibody responses differed among the tested antigens. Totally, 87/158 patients were HPV positive with PCR at some occasion, of which 49 were HPV negative at enrolment but became positive during follow-up. A sample of these patients were studied to elucidate the sensitivity and type-specificity of the serological assays. A common pattern for the antibody response was a "transient peak", i.e. return of the antibody levels to levels

close to these before infection after disappearance of the viral DNA. The majority of patients with new HPV DNA showed an induction of HPV antibodies, either as a transiently induced antibody peak or as a continuous induction. The HPV type-specificity of the antibody responses was, however, limited, since most HPV antibodies were induced by infection with several HPV types (VI).

In paper VII, we studied the development of the HPV antibody response among 26 patients treated for condylomata. The patients were seen at the clinic a mean of 4.5 times and received a mean of 1.9 chemical and 4.8 surgical treatments. The mean length of the follow-up was 8.4 months. Only 8/26 (31%) patients were cured during follow-up. Besides the peptide antigens and the HPV 16 capsids that were used in the previous studies, we also studied the antibody response against HPV 6 and 11 capsids. For several patients there were dramatic changes in antibody responses during follow-up. The responses did not differ between the cured and the non-cured group. The most abundant antibody responses were noted for IgA and IgG to HPV 6 capsids. Whereas IgG frequently increased (transiently or continuously) during follow-up, IgM usually declined. The antibody responses against HPV 6 and 11 capsids as well as those against HPV 6 and 16 capsids, were related, suggesting the existence of cross-reactivity. The antibody responses to HPV 11 and HPV 16 capsids were not related.

We also studied whether acquisition of HPV 6 infection was associated with the development of antibodies to HPV 6 capsids (that had not been available in the previous study VI). For this purpose, we used the same cohort of subjects with multiple sexual partners that was also studied in the previous seroconversion study; we identified 17 patients with an HPV 6 infection detected during follow-up, but who were HPV-negative at enrolment. For 12/17 subjects there were transiently induced antibody peaks or continuous inductions of HPV 6 capsid antibodies concomitantly with HPV 6 DNA detection, although 8/17 patients only developed measurable levels of antibodies of one antibody class (IgA, IgG or IgM). The induction of HPV 6 capsid antibodies concomitantly with HPV DNA detection was highly statistically significant ($p=0.002$).

DISCUSSION

Available treatment modalities for GPVI include chemical (local application of podophyllin/podophyllotoxin) and surgical (diathermy/laser evaporation) methods. Recurrence rates of 30-40% (26, 345) for podophyllotoxin, and in up to 25% using surgical therapy (144, 295), have been described. Hence, no completely effective therapy exists for genital warts. In Sweden local self-treatment with podophyllotoxin is commonly used as the first choice of therapy against penile warts in the majority of cases, and is often prescribed by the primary care physician. Yet, a number of patients still need specialist referral for surgical therapy performed by venereologists, gynecologists and sometimes also urologists and proctologists.

In order to evaluate the effectiveness of available therapy modalities in men, we performed two retrospective studies (I, IV). The majority of patients became wart-free after a few therapeutical sessions from chemotherapy and/or surgery; the cure rate after 1-4 sessions was as high as 82%. Yet, a fraction of the patients required as much as 5-12 therapy sessions (I). This study was not designed to compare efficacy from chemotherapy versus surgical methods. Surgery was frequently given as primary therapy on locations that empirically do not respond very well to podophyllotoxin (336), such as in the meatus urethrae, on the penile shaft and in the anal area. Primary surgery was also used on a relatively wide indication during the study because of ongoing collection of wart tissue for HPV DNA analysis (309, 344).

A group of condyloma patients referred to a specialized STD clinic will inevitably represent "problem cases" either because they exhibit a high degree of recalcitrance to topical chemotherapy, or they attend due to a relatively large wart bulk and/or multifocal wart growth. In our material as much as 20% of patients were still not cured within one year (I). Our figures for cure rates are based on a retrospective follow-up investigation based on the presumption that patients were cured if they were wart-free at one

visit after the last therapy had been given. Thus, our estimate on efficacy may possibly represent an overestimation, the true magnitude of therapeutic recalcitrance being even higher than accounted for by us. However, although the risk of wart recurrence generally appears to be highest the first 3-6 months after cure first is assumed (336), additional cases of recurrence may certainly occur later on. Accordingly, it cannot be excluded that some of the patients appraised by us as "cured" might subsequently have had wart recurrences. On the other hand, these "recurrences" may also represent "re-occurrences" (i.e. warts occurring on sites previously not submitted to therapy), either due to reactivation of latent and/or subclinical infection, or as a result of new HPV infection(s).

As concluded in the two studies on therapeutic efficacy (I, IV), the occurrence of warts in the perianal area and/or in the anus is not uncommon among heterosexual men. However, intraanal warts are significantly more common in the homosexual group (I, IV). Among patients with warts in the anal region, the existence of concurrent penile warts is, on the other hand, more common among heterosexual men; suggestive that GPVI in heterosexual men may be acquired by autoinoculation by the patient´s fingers, from penile warts to the anal region (I, IV). The difference in number of therapeutic sessions and the time required until accomplished cure between the heterosexual and homosexual study population is striking (IV). An evident explanation is the multifocal expression per se, requiring repetitive treatment for both penile and anal warts in the heterosexual men. An alternative and highly likely interpretation includes that the multifocal development of warts reflects an associated default in the function of HPV specific immunological surveillance mechanisms. This hypothesis might also, to some degree, account for the relatively high degree of therapeutic recalcitrance in the heterosexual study population. The validity of this premise might seem contradictory to the finding that no difference was detected in therapy outcome between HIV-positive and -negative men. However, it should be emphasized that, first the number of HIV-positive men in our study was low; furthermore the general level of immunological function in the HIV-infected men should be regarded as being relatively intact, with CD4 counts being in the range of 220-960 (mean 524).

Accordingly, it is not surprising that a low detection rate of AIN existed in these men; reported risk of AIN among HIV positive men appears to occur relatively late in the HIV infection (205).

Anal cancer is a malignancy that is 2-3 times more common in females and has a peak incidence at about 65 years of age (112). Reports from the San Francisco Bay area, have also detected an increased risk for this cancer among homosexual men, also in younger ages. Although most prominent among the HIV infected men, an increased risk has also been seen in the men with intact cellular immune function. A strong association between a history of genital warts, AIN and subsequent induction of anal malignancies has been reported (205, 221). It is recommended that anal cytology is taken routinely among HIV-infected men with CD4-values below 200 (232).

A major draw-back of available methods is that they can only eradicate visible lesions. To eliminate the risk of future recurrences, a therapeutic method that eliminates the viral persistence would be preferrable. A possible way to achieve this goal is the use of immunomodulatory drugs or therapeutic vaccines to stimulate the immune response.

There are several explanations for the low degree of HPV positivity detected by us in acetowhite lesions; merely 49% of the samples were SB positive and 19% ISH positive. We believe that one major reason for the low positivity rates is that rather small biopsies were collected. Also, a low number of viral copies in acetowhite lesions may also have contributed. The presence of HPV DNA in acetowhite lesions also varies between different studies dependent on hybridisation methods used and colposcopic criteria applied, but seldom exceeds 50% ((16, 30, 59, 131, 273, 278), paper II), compared to 80-90% of classical warts ((344), paper IV). In one study on subclinical vulval lesions, Cone et al (59) found that as few as 14% of biopsies were HPV positive. An additional explanation might be that other HPV types than the ones tested for were present in the lesions.

In our study on penoscopic criteria for acetowhite GPVI lesions (II), koilocytosis - the pathognomonic hallmark of productive nuclear viral replication and most commonly occurring in classical condylomas (257) - was detected in as much as 48% of biopsies, in concordance with previous reports using the PCR-in situ technique, showing that HPV DNA is often detectable in cells lacking koilocytosis (222). It was not unexpected that PCR analysis gave the highest sensitivity for viral influence (72%) in study II. Accordingly, the finding of PCR negativity in all of the ISH negative lesions in study III, focusing on samples collected from men with recurrent balanoposthitis, seems rather startling. However, differences in the PCR technique used in the two studies may account for the incongruity. Thus, first paraffin embedded tissue was used in study III, while freshly frozen biopsies were analysed in study II. Furthermore, we used less sensitive consensus primers in study III rather than type-specific primers used in study II.

The histological finding of normal skin in as much as 5/91 (5%) and other diagnoses in another 3% of acetowhite lesions elucidates a lack of specificity of the acetic acid test for the detection of GPVI lesions. We advocate that the use of the test always should be combined with colposcopic magnification and use of strict morphological criteria for identification of underlying GPVI. A directed biopsy is usually indicated for light microscopic verification due to a high frequency of false positivity of the acetic acid test.

Inflammatory responses existing concurrently with GPVI does not seem to be uncommon. An inflammatory component was found in 25% of the biopsies from subclinical lesions (II), and in 74% of lesions sampled from cases classified as HPV-associated balanoposthitis (III). Apparently, an inflammatory response occurs in asymptomatic (II) as well as in patients with recurrent and long-lasting symptoms such as itching, burning and dyspareunia (III). It is possible that, in some cases, the inflammation represents a favourable prognostic marker in analogy with the finding of dermal and/or epidermal lymphocyte and macrophage accumulation seen in spontaneously regressing cutaneous warts (140).

As being apparent by penoscopic as well as by electron microscopic magnification, acetowhite lesions may exhibit epidermal fissures, which not only might be of a pathogenetic significance for inducing local symptoms per se, but also might serve as entrances for other microbiological agents that could contribute to the development of local inflammatory responses. Secondary inflammatory reactions due to, for example candida infection, may also induce false positive acetowhite reactions ((273), paper II). In recent years a potential synergistic effect by candida infections has been discussed as a contributing factor for GPVI associated symptomatology, in particular for vulvar lesion (200). As emphasized in study II, we recommend that non-specific acetowhite reaction patterns are identified, and appropriate therapy given for underlying inflammatory conditions prior to therapy of the GPVI lesions.

The 23 cases analysed in study III reveal obvious similarities with the condition previously described in females as "papillomavirus vulvovaginitis" (29). We propose that, in men, the analogous term "papillomavirus-associated balanoposthitis" is applied for a clinical condition that has hardly been recognized clinically previously. Although the condition probably rather rarely is associated with long-lasting morbidity, we would like to emphasize that in cases of balanoposthitis of unknown etiology, the possibility of an underlying GPVI should be kept in mind.

In the first serological study (V), we found that patients with previous condylomata had significantly increased antibody levels for IgG to peptide antigens derived from the L1 and L2 capsid proteins of HPV 6, compared to controls. However, the antibody response among patients with visible condylomata did not differ from controls, possibly because our control group consisted of patients attending an STD clinic, a "high risk group" for STD including GPVI. Our unability to detect these antibodies among patients with visible condylomata might also be due to a slow development of the antibody response against these epitopes during disease, similar to the slow development of serological response to cutaneous HPVs (64). However, the response against assembled HPV 6 capsids was not remarkably slow among patients with new HPV infection (VII). It usually occurred at the same visit

as HPV DNA was detected, or at least the visit after. As the mean interval between two visits was six months, we can only conclude that the response did develop within this time. It is possible that the patients with visible warts in study V, had sought medical attention shortly after being afflicted with warts such that a serological response did not have time to develop, although the choice of control group is the most plausible explanation. In a study similar to ours, Jenison et al (143) used recombinant proteins in Western blot for the detection of antibodies to various HPV antigens; they did not find any significant differences in antibody responses among STD patients compared to children. This lack of difference, is probably due to a low degree of HPV type specificity in their serological assays. When a multitude of epitopes are exposed in the assays based on recombinant proteins, it is difficult to know to which epitope(s) the antibodies measured are directed (77).

In the study of HPV seroconversions (VI), we showed that patients who were seronegative at enrolment but later seroconverted, became seropositive for several antigens at the same visit. This usually occurred at the same visit as HPV DNA was detected or in some cases the visit after. The HPV type specificity of the serological assays was limited; antibody responses were induced by several different HPV types. The most type-restricted response was the response against HPV 16 capsids which was induced at higher levels and tended to continuously increase after infection with HPV type 16, compared to infection with other HPV types. The vast majority of the infections among the asymptomatic patients were transient, being detected only at a single occasion. Our results are thus in concordance with the study by Evander et al (95). The antibody response against transient genital HPV infection was frequently also transient, a finding that will complicate attempts to determine total lifetime exposure to HPV by seroepidemiology. Our identification of HPV seroconversions has also enabled the postulation of a new specificity criterium for evaluating HPV-serological assays. Specific antibodies are induced at the time of a seroconversion occurring concomitantly with acquisition of HPV DNA. The specificity criteria previously used are that antibodies are preferentially found among patients with HPV-associated disease

((78, 177, 180, 249), paper V), that seropositivity against several antigens is found in the same patients ((77, 81), paper VI), and that antibody titers decrease following regression or treatment of HPV-associated lesions (77, 180).

SUMMARY

Efficacy of chemical and/or surgical treatment for penile and anal condylomata acuminata was investigated in two retrospective studies of hetero- and homosexual men. Variation in clinical features and symptomatology as well as the reliability of diagnostic criteria by different methods for acetowhite penile lesions was also studied. Furthermore, the antibody response in the course of penile wart disease as well as in asymptomatic genitoanal papillomavirus infection (GPVI) was analysed.

In the first retrospective study (I), as much as 23% of patients still had condylomas after one year of chemical and/or surgical treatment. On the other hand, 38% were cured after a single treatment session. In the group mainly with anal warts (IV), concurrent penile warts were significantly more common among heterosexual men compared to homosexual men ($p<0.001$), while intra-anal wart growth was more common among the homosexual males ($p<0.001$). When comparing diagnostic methods for subclinical penile HPV infection, conventional histopathology appeared to be the most valuable diagnostic aid to penoscopy, while the additional use of Southern blot, in situ hybridisation and PCR assays for HPV DNA detection did not increase the predictive value of GPVI (II). We also describe a new distinct clinical entity, HPV-associated balanoposthitis, comprising a wide range of often long-lasting symptoms, such as itching, burning and dyspareunia (III).

A significant increase in the IgG antibody response against defined epitopes in the L1 and L2 capsid proteins of HPV 6, was found among men with previous condylomata (V). By following a cohort of STD clinic patients with multiple brush samples from the genitoanal region as well as serum samples taken at several consecutive clinical visits, we identified 16 patients who had seroconverted to HPV seropositivity during follow-up (VI). Antibody responses to several HPV-derived peptide and protein antigens were induced at the same time. Seroconversions were usually seen concomitantly with HPV acquisition or at the visit after HPV DNA was first detected (VI). The HPV antibody response was frequently transient

and declined or disappeared after clearance of infection. The antibody responses were induced by several different HPV types, indicating limited type-specificity (VI, VII). The most type-restricted response was against HPV 16 capsids, where seroconversions to continuous seropositivity were induced by infection with HPV 16.

ACKNOWLEDGEMENTS

I want to express my sincere gratitude to:

Geo von Krogh, my "clinical" tutor for introducing me into science and the exciting papillomavirus field, his enthusiasm, friendship and support.

Joakim Dillner, my "virological" tutor for his great scientific knowledge, interesting discussions late in the evenings, and always being ready to help.

Professor Sture Lidén at the Dept. of Dermatovenereology for his support and for giving me the opportunity to conduct research.

Professor Erling Norrby at the Dept. of Virology for letting me perform the experimental part of the thesis at the Dept. of Virology.

Carina Eklund for her excellent technical skills and invaluable help in the laboratory.

Vanoohi Fredriksson-Shanazarian and Marika Stenbeck for professional technical assistence.

The staff at the Dept. of Dermatovenereology (especially the nurses Elisabeth Klingsell, Staffan Lidén and Anna-Kerstin Andersson) for their help with the blood samples.

The colleagues at the Dept. of Dermatovenereology and Virology for pleasent company.

My co-authors for fruitful collaborations.

My parents and friends for encouragement and support.

All patients at the STD clinic who consented to donate serum samples, and without whom this thesis would not have been possible.

These studies were supported by grants from Edvard Welanders stiftelse, KI fonder and Cancerfonden.

REFERENCES

1. Ackerman AB, Kornberg R. Pearly penile papules. Arch Dermatol 1973;108:673-675.

2. Agarwal SS, Sehgal A, Sardana S, Kumar A, Luthra UK. Role of male behaviour in cervical carcinogenesis among women with one lifetime sexual partner. Cancer 1993;72:1666-1669.

3. Almeida JD, Goffe AP. Antibody to wart virus in human sera demonstrated by electron microscopy and precipitin tests. Lancet 1965;i:1205.

4. Andersson-Ellström A, Dillner J, Hagmar B, Schiller J, Forssman L. No serological evidence for non-sexual spread of HPV 16. Lancet 1994;344:i:1435.

5. Ansink AC, Krul MRL, de Weger RA, Kleyne JAFW, Pupers H, van Tintern H, de Kraker EW, Helmerhorst TJM, Heintz APM. Human papillomavirus, lichen sclerosus and squamous cell carcinoma of the vulva: detection and prognostic significance. Gynecol Oncol 1994;52:180-184.

6. Anwar K, Phil M, Naiki H, Nakakuki K, Inuzuka M. High frequency of human papillomavirus infection in carcinoma of the urinary bladder. Cancer 1992;70:1967-1973.

7. Apple RJ, Erlich HA, Klitz W, Manos MM, Becker TM, Wheeler CM. HLA DR-DQ associations with cervical carcinoma show papillomavirus-type specificity. Nat Genet 1994;6:157-162.

8. Arends MJ, Donaldson YK, Duvall E, Wyllie AH, Bird CC. Human papillomavirus type 18 associates with more advanced cervical neoplasia than human papillomavirus type 16. Hum Pathol 1993;24:432-437.

9. Association ASH. Survey shows how we live with HPV. HPV News 1993;3:1-9.

10. Baefverstedt B. Condylomata acuminata - past and present. Acta Dermatol Venereol 1967;47:376-381.

11. Baggish MS. Improved laser techniques for the elimination of genital and extragenital warts. Am J Obstet Gynecol 1985;153:545-550.

12. Baker CC. Sequence analysis of papillomavirus genomes. In: "The papillomaviridae" N Salzman & P Howley, Eds Plenum Press, New York 1987;2:321-385.

13. Baker DA, Douglas JM, Buntin DM, Micha JP, Beutner KR, Patsner B. Topical podofilox for the treatment of condylomata acuminata in women. Obstet Gynecol 1990;76:656-659.
14. Bar-Am A, Shilon M, Peyser MR, Ophir J, Brenner S. Treatment of male genital condylomatous lesions by carbon dioxide laser after failure of previous nonlaser methods. J Am Acad Dermatol 1991;24:87-89.
15. Barr BBB, Benton EC, McLaren K, Bunney MH, Smith IW, Blessing K, Hunter JAA. Human papillomavirus infection and skin cancer in renal allograft recipients. Lancet 1989;ii:124-128.
16. Barrasso R, DeBrux J, Croissant O, Orth G. High prevalence of papilloma virus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia. N Engl J Med 1987;317:916-923.
17. Bauer HM, Ting Y, Greer CE, Chambers JC, Tashiro CJ, Chimera J, Reingold A, Manos MM. Genital human papillomavirus infection in female university students as determined by a PCR-based method. J Am Med Ass 1991;265:472-477.
18. Bedell MA, Hudson JB, Golub TR, Turyk ME, Hosken M, Wilbanks GD, Laimins LA. Amplification of human papillomavirus genomes in vitro is dependent on epithelial differentiation. J Virol 1991;65:2254-2260.
19. Bell B. Treatise on gonorrhoea virulenta and lues venerea. Edinburgh 1793;1:421-441.
20. Bellina JH. The use of the carbon dioxide laser in the management of condyloma acuminatum with eight-year follow-up. Am J Obstet Gynecol 1983;147:375-378.
21. Benedet JL, Miller DM, McKerson KG, Anderson GH. The results of cryosurgical treatment of cervical intraepithelial neoplasia at one, five and ten years. Am J Obstet Gynecol 1987;157:268-273.
22. Bergman A, Matsunaga J, Bhatia N. Cervical cryotherapy for condylomata acuminata during pregnancy. Obstet Gynecol 1987;69:47-50.
23. Bergman A, Nalick R. Genital human papillomavirus infection in men. Diagnosis and treatment with a laser and 5-fluorouracil. J Reprod Med 1991;36:363-366.
24. Berman A, Winkelmann RK. Flat warts undergoing involution. Histopathological findings. Arch Dermatol 1977;113:1219-1221.

25. Bernard BA, Bailly C, Lenoir M-C, Darmon M, Thierry F, Yaniv M. The human papillomavirus type 18 (HPV 18) E2 gene product is a repressor of the HPV 18 regulatory gene in human keratinocytes. J Virol 1989;63:4317-4724.
26. Beutner KR, Conant MA, Friedman-Kien A, Illeman M, Artman NN, Thisted RA, King DH. Patient-applied podofilox for treatment of genital warts. Lancet 1989;ii:831-834.
27. Birley HDL, Luzzi GA, Walker MM, Ryait B, Taylor-Robinson D, Renton AM. The association of human papillomavirus infection with balanoposthitis: A description of five cases with proposals for treatment. Int J STD & AIDS 1994;5:139-141.
28. Björge T, Hennig EM, Baadstrand Skare G, Söreide O, Österbö Thoresen S. Second primary cancers in patients with carcinoma in situ of the uterine cervix. The Norwegian experience 1970-1992. Int J Cancer 1995;In press.
29. Bodén E, Eriksson A, Rylander E, von Schoultz B. Clinical characteristics of papillomavirus-vulvovaginitis. Acta Obstet Gynecol Scand 1988;67:147-151.
30. Bodén E, Rylander E, Evander M, Wadell G, von Schoultz B. Papillomavirus infection of the vulva. Acta Obstet Gynecol Scand 1989;68:179-184.
31. Bonnez W, Da Rin C, Rose RC, Reichman RC. Use of human papillomavirus type 11 virions in an ELISA to detect specific antibodies in humans with condyloma acuminata. J Gen Virol 1991;72:1343-1347.
32. Bonnez W, Da-Rin C, Rose RC, Tyring SK, Reichman RC. Evolution of the antibody response to human papillomavirus type 11 (HPV-11) in patients with condyloma acuminatum according to treatment response. J Med Virol 1993;39:340-344.
33. Bosch FX, Munoz N, DeSanjose S, Izarzugaza I, Gili M, Viladice P, Tormo MJ, Moreo P, Ascunce N, Gonzalez LC, Tafur L, Kalder JM, Guerrero E, Aristizabal N, Santamaria M, Alonso de Ruiz P, Shah K. Risk factors for cervical cancer in Colombia and Spain. Int J Cancer 1992;52:750-758.
34. Brandsma JL, Abramson AL. Association of papillomavirus with cancers of the head and neck. Arch Otolaryngol Head Neck Surg 1989;115:621-625.
35. Braun J, Raguse T. The value of surgical excision in the treatment of anal condylomas. Colo-proctol 1987;9:23-26.

36. Brinton LA, Fraumeni Jr JF. Epidemiology of uterine cervical cancer. J Chron Dis 1986;39:1051-1065.
37. Brinton LA, Nasca PC, Mallin K, Baptiste MS, Wilbanks GD, Richart RM. Case-control study of cancer of the vulva. Obstet Gynecol 1990;75:859-866.
38. Brinton LA, Reeves WC, Brenes MM, Herrero R, de Britton RC, Gaiton E, Tenorio F, Garcia M, Rawls WE. Oral contraceptive use and risk of invasive cervical cancer. Int J Epidemiol 1990;19:4-11.
39. Bryant P, Davies P, Wilson D. Detection of human papillomavirus DNA in cancer of the urinary bladder by in situ hybridisation. Br J Urol 1991;68:49-52.
40. Bunney MH, Benton C, Cubie HA. Viral warts Biology and treatment Oxford University Press, Oxford 1992.
41. Burkett BJ, Peterson CM, Birch LM, Brennan C, Nuckols ML, Ward BE, Crum CP. The relationship between contraceptives, sexual practices, and cervical human papillomavirus infection among a college population. J Clin Epidemiol 1992;45:1295-1302.
42. Byrne P, Woodman C, Kelly K. How accurate is colposcopy in the diagnosis of cervical human papillomavirus infection? J Obstet Gynecol 1988;9:60-64.
43. Campion MJ, Singer A, Clarkson PK, McCance DJ. Increased risk of cervical neoplasia in consorts of men with penile condylomata acuminata. Lancet 1985;ii:943-946.
44. Candorin MJ, Sopracordevole F, Iuzzolino C, Tonetto G, Nenzi F. Histological diagnosis of human papillomavirus (HPV)-related dysplasia: quality control by in situ hybridization (ISH) analysis. Eur J Gyneacol Oncol 1992;13:427.
45. Carpiniello VL, Zderic SA, Malloy TR, Sedlacek T. Carbon dioxide laser therapy of subclinical condyloma found by magnified penile surface scanning. Urology 1987;29:608-610.
46. Cecchini S, Cipparrone I, Confortini M, Scuderi L, Meini L, Piazzesi G. Urethral cytology of cytobrush specimens. A new technique for detecting subclinical human papillomavirus infection in men. Acta Cytologica 1988;32:314-317.
47. Celentano DD, Klassen AC, Weisman CS, Rosenhein NB. The role of contraceptive use in cervical cancer: The Maryland cervical case-control study. Am J Epidemiol 1987;126:592-604.

48. Chang F, Syrjänen S, Kellokoski J, Syrjänen K. Human papillomavirus (HPV) infections and their associations with oral disease. J Oral Pathol Med 1991;20:305-317.
49. Chang F, Syrjänen S, Shen Q, Ji H, Syrjänen K. Human papillomavirus (HPV) DNA in esophageal precancer lesions and squamous cell carcinomas from China. Int J Cancer 1990;45:21-25.
50. Chang F, Wang L, Syrjänen S, Syrjänen K. Human papillomavirus infections in the respiratory tract. Am J Otolaryngol 1992;13:210-225.
51. Chellappan SP, Hiebert S, Mudryj M, Horowitz JM, Nevins JR. The E2F transcription factor is a cellular target for the RB protein. Cell 1991;65:1053-1061.
52. Chen S, Slavin J, Fairley CK, Tabrizi SN, Borg AJ, Billson V, Garland SM. The absence of HPV DNA in genital specimens from infants. Genitourin Med 1993;69:270-272.
53. Chetsanga C, Malmström P-U, Gyllensten U, Moreno-Lopez J, Dinter Z, Pettersson U. Low incidence of human papillomavirus type 16 in bladder tumor detected by the polymerase chain reaction. Cancer 1992;69:1208-1211.
54. Chiang CM, Ustav M, Stenlund A, Ho TF, Broker TR, Chow LT. Viral E1 and E2 proteins support replication of homologous and heterologous papillomavirus origins. Proc Natl Acad Sci 1992;89:5799-5803.
55. Chuang T-Y, Perry HO, Kurland LT, Ilstrup DM. Condyloma acuminatum in Rochester, Minn, 1950-1978. I. Epidemiology and clinical features. Arch Dermatol 1984;120:469-475.
56. Ciuffo G. Innesto positivo con filtrato di verrucae volgare. Giorn Ital Mal Venereol 1907;48:12-17.
57. Clarke EA, Hatcher J, McKeown-Eyssen GE, Lickrish GM. Cervical dysplasia: association with sexual behaviour, smoking, and oral contraceptive use? Am J Obstet Gynecol 1985;5:612-616.
58. American College of Obstreticians and Gynecologists gynecologic practice committee. Guidelines recommended to protect against viruses convoyed in laser smoke. ACOG Newslett 1989;33:18.
59. Cone R, Beckmann A, Aho M, Wahlström T, Ek M, Corey L, Paavonen J. Subclinical manifestations of vulvar human papillomavirus infection. Int J Gynecol Pathol 1991;10:26-35.

60. Cook LS, Koutsky LA, Holmes KK. Clinical presentation of genital warts among circumcised and uncircumcised heterosexual men attending an urban STD clinic. Genitourin Med 1993;69:262-264.
61. Cripe TP, Haugen TH, Turk JP, Tabatabai F, Schmid PG, Durst M, Gissmann L, Roman A, Turek LP. Transcriptional regulation of the human papillomavirus 16 E6-E7 promoter by a keratinocyte-dependent enhancer and by viral E2 transactivator and repressor gene products; implications for cervical carcinogenesis. EMBO J 1987;6:3745-3753.
62. Crook T, Wrede D, Vousden K. p53 point mutation in HPV negative human cervical carcinoma cell lines. Oncogene 1991;6:873-875.
63. 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.
64. Cubie HA. Serological studies in a student population prone to infection with human papillomavirus. J Hyg Camb 1972;70:677-690.
65. Culp OS, Kaplan IW. Condylomata acuminata: 200 cases treated with podophyllin. Ann Surg 1944;120:251-256.
66. Damstra RJ, van Vloten WA. Cryotherapy in the treatment of condylomata acuminata: A controlled study of 64 patients. J Dermatol Surg Oncol 1991;17:273-276.
67. Davidson-Parker J, Dinsmore W, Khan MH, Hicks DA, Morris CA, Morris DF. Immunotherapy of genital warts with inosine pranobex and conventional treatment: double blind placebo controlled study. Genitourin Med 1988;64:383-386.
68. Day NE. Screening for squamous cervical cancer: duration of low risk after negative results of cervical cytology and its implication for screening policies. BMJ 1986;293:659-664.
69. de Roda Husman A-M, Walboomers JMM, Meijer CJLM, Risse EKJ, Schipper MEI, Helmerhorst TM, Bleker OP, Delius H, van den Brule AJC, Snijders PJF. Analysis of cytomorphologically abnormal cervical scrapes for the presence of 27 mucosotropic human papillomavirus genotypes, using polymerase chain reaction. Int J Cancer 1994;56:802-806.
70. de Sanjosé S, Munoz N, Bosch FX, Reijmann K, Pedersen NS, Orfila J, Ascunce N, González LC, Tafur L, Gili M, Lette I, Viladiu P, Tormo MJ, Moreo P, Shah K, Wahren B. Sexually transmitted agents and

cervical neoplasia in Colombia and Spain. Int J Cancer 1994;56:358-363.
71. de Villiers E-M. Heterogeneity of the human papillomavirus group. J Virol 1989;63:4898-4903.
72. de Villiers E-M. Human pathogenic papillomavirus types: An update. Curr Top Microbiol Immunol 1994;186:1-12.
73. de Villiers EM. Laboratory techniques in the investigation of human papillomavirus infection. Genitourin Med 1992;68:50-54.
74. Debenedictis TJ, Marmar JL, Praiss DE. Intraurethral condylomas acuminata: management and review of the literature. J Urol 1977;118:767-769.
75. Del Mistro A, Koss LG, Braunstein J, Bennet B, Saccomano G, Simons KM. Condylomata acuminata of the urinary bladder. Amer J Surg Pathol 1988;12:205-215.
76. Dillner J. Mapping of linear epitopes of the human papillomavirus type 16: The E1,E2, E4, E5, E6 and E7 open reading frames. Int J Cancer 1990;46:703-711.
77. Dillner J. Antibody responses to defined HPV epitopes in cervical neoplasia. Papillomavirus Report 1994;5:35-41.
78. Dillner J, Dillner L, Robb J, Willems J, Jones I, Lancaster W, Smith R, Lerner R. A synthetic peptide defines a serologic IgA response to a human papillomavirus-carrying cervical neoplasia. Proc Natl Acad Sci 1989;86:3838-3841.
79. Dillner J, Dillner L, Utter G, Eklund C, Rotola A, Costa S, DiLuca D. Mapping of linear epitopes of human papillomavirus type 16: the L1 and L2 open reading frames. Int J Cancer 1990;45:529-535.
80. Dillner J, Knekt P, Schiller JT, Hakulinen T. Prospective seroepidemiological evidence that human papillomavirus type 16 infection is a risk factor for esophageal cancer. 1995. Manuscript.
81. Dillner J, Lenner P, Lehtinen M, Eklund C, Heino P, Wiklund F, Hallmans G, Stendahl U. A population-based seroepidemiological study of cervical cancer. Cancer Res 1994;54:134-141.
82. Dillner J, Wiklund F, Lenner P, Eklund C, Fredriksson-Shanazarian V, Schiller JT, Hibma M, Hallmans G, Stendahl U. Antibodies against linear and conformational epitopes of human papillomavirus type 16 that independently associate strongly with cervical cancer. Int J Cancer 1995;60:1-6.
83. Dillner L, Fredriksson A, Persson E, Forslund O, Hansson BG, Dillner J. Antibodies against papillomavirus antigens in cervical

secretions from condyloma patients. J Clin Microbiol 1993;31:192-197.
84. Dillner L, Zellbi A, Åvall E, Heino P, Eklund C, Pettersson C-A, Forslund O, Hansson B-G, Grandien M, Bistoletti P, Dillner J. Association of serum antibodies against defined epitopes of human papillomavirus L1, E2 and E7 antigens and of HPV DNA with incident cervical cancer. Cancer Detect Prev 1995.In press.
85. Doorbar J, Campbell D, Grand R, Gallimore P. Identification of the human papillomavirus 1a E4 gene products. EMBO J 1986;5:355-362.
86. Doorbar J, Ely S, Sterling J, McLean C, Crawford L. Specific interactions between HPV 16 E1-E4 and cytokeratins results in collapse of the epithelial intermediate filament networks. Nature 1991;352:824-827.
87. Duus BR, Philipsen T, Christensen JD, Lundvall F, Söndergaard J. Refractory condylomata acuminata: a controlled clinical trial of carbon dioxide laser versus conventional surgical treatment. Genitourin Med 1985;61:59-61.
88. Dyall-Smith D, Trowell H, Dyall-Smith ML. Benign human papillomavirus infection in renal transplant recipient. Int J Dermatol 1991;30:785-789.
89. Dyson N, Howley PM, Munger K, Harlow E. The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 1989;243:934-937.
90. Edwards A, Atma-Ram A, Thin RN. Podophyllotoxin 0.5% versus podophyllin 20% to treat penile warts. Genitourin Med 1988;64:263-265.
91. Elfgren K, Bistoletti P, Dillner L, Dillner J. Serum and cervical secretion antibody levels against HPV-derived antigens regularly decline following treatment for CIN III. 1995.Manuscript.
92. Eliezri YD, Silverstein SJ, Nuovo GJ. The occurrence of human papillomavirus DNA in cutaneous squamous and basal cell neoplasms. J Am Acad Dermatol 1990;23:836.
93. Euvrard S, Chardonnet Y, Pouteil-Noble C, Kanitakis J, Chignol MC, Thivolet C, Touraine JL. Association of skin malignancies with various and multiple carcinogenic and noncarcinogenic human papillomaviruses in renal transplant recipients. Cancer 1993;72:2198-2206.

94. Euvrard S, Chardonnet Y, Pouteil-Noble J, Kanitakis J, Thivolet J, Touraine JL. Skin malignancies and human papillomaviruses in renal transplant recipients. Transplant Proc 1993;25:1392-1393.
95. Evander M, Edlund K, Gustafsson Å, Jonsson M, Karlsson R, Rylander E, Wadell G. Human papillomavirus infection is transient in young women: A population-based cohort study. J Infect Dis 1995.In press.
96. Evans AS, Monaghan JM. Spontaneous resolution of cervical warty dysplasia: The relevance of clinical and nuclear DNA features: A prospective study. Br J Obstet Gynecol 1985;92:165.
97. Fairley CK, Chen S, Tabrizi SN, Leeton K, Quinn MA, Garland SM. The absence of genital human papillomavirus DNA in virginal women. Int J STD & AIDS 1992;3:414-417.
98. Ferenczy A. Laser treatment of genital human papillomvirus infections in the male patient. Obstet Gynecol Clin North Amer 1991;18:525-535.
99. Ferenczy A, Bergeron C, Richart RM. Human papillomavirus DNA in CO2 laser-generated plume of smoke and its consequences to the surgeron. Obstet Gynecol 1990;75:114-118.
100. Ferenczy A, Mitao M, Nagai N, Silverstein, SJ, Crum CP. Latent papillomavirus and recurring genital warts. N Engl J Med 1985;313:784-788.
101. Filiberti A, Tamburini M, Stefanon B, Merola M, Bandieramonte G, Ventafridda B, De Palo G. Psychological aspects of genital human papillomavirus infection: a preliminary report. J Psychosom Obstet Gynecol 1993;14:145-152.
102. Finlay CA, Hinds PW, Levine AJ. The p53 protooncogene can act as a suppressor of transformation. Cell 1989;57:1083-1093.
103. Fisher AA. Severe systemic and local reaction to topical podophyllum resin. Cutis 1981;28:233-266.
104. Fisher M, Rosenfeld WD, Burk RD. Cervicovaginal human papillomavirus infection in suburban adolescents and young adults. J Pediatr 1991;119:821-825.
105. Fletcher JL. Perinatal transmission of human papillomavirus. AFP 1991;43:143-148.
106. Franco EL. The sexually transmitted disease model for cervical cancer: incoherent epidemiologic findings and the role of misclassification of human papillomavirus infection. Epidemiology 1991;2:98-106.

107. Frazer IH, Leonard JH, Schonrock J, Wright RG, Kearsley JH. HPV DNA in oropharyngeal squamous cell cancers: comparison of results from four DNA detection methods. Pathology 1993;25:138-143.
108. Frisch M, Melbye M, Moller H. Trends in incidence of anal cancer in Denmark. BMJ 1993;306:419-422.
109. Galloway DA, Jenison SA. Characterization of the humoral immune response to human genital papillomaviruses. Mol Biol Med 1990;7:59-68.
110. Gemy. Quoted Frey E (1924). Schweiz Med Wochenschr 1893;5:215-219.
111. Glinski W, Obalek S, Jablonska S, Orth G. T cell defect in patients with epidermodysplasia verruciformis due to human papillomaviruses types 3 and 5. Dermatologica 1981;162:141-147.
112. Goldman S, Glimelius B, Nilsson B, Paahlman L. Incidence of anal epidermoid carcinoma in Sweden 1970-1984. Acta Chir Scand 1989;155:191-197.
113. Goldman S, Glimenius B, Påhlman L, Ståhle E, Wilander E. Anal epidermoid carcinoma: a population-based clinicopathological study of 164 patients. Int J Colorect Dis 1988;3:109-118.
114. Goorney BP, Waugh MA, Clarke J. Anal warts in heterosexual men. Genitourin Med 1987;63:216.
115. Greene I. Therapy for genital warts. Dermatol Clin 1992;10:253-267.
116. Gross G, Hagedorn M, Ikenberg H, Rufli T, Dahlet C, Grosshans E, Gissmann L. Bowenoid papulosis. Presence of human papillomavirus structural antigens and of HPV-16-related DNA sequences. Arch Dermatol 1985;121:858-863.
117. Grussendorf-Conen E-I, Meinhof W, de Villiers E-M, Gissman L. Occurrence of HPV genomes in penile smears of healthy men. Arch Dermatol Res 1987;279:S73-S75.
118. Guerrero E, Shah K. Polymerase chain reaction in HPV diagnosis. Papillomavirus Report 1991;2:115-118.
119. Gutman LT, Herman-Giddens ME, Phelps WC. Transmission of human genital papillomavirus disease: comparison of data from adults and children. Pediatrics 1993;91:31-38.
120. Hallmo P, Naess O. Laryngeal papillomatosis with human papillomavirus DNA contracted by a laser surgeon. Eur Arch Otorhinolaryngol 1991;248:425-427.

121. Happonen H-P, Lassus A, Santalahti J, Forsström S, Lassus J. Topical idoxuridine for treatment of genital warts in males. A double-blind comparative study of 0.25% and 0.5% cream. Genitourin Med 1990;66:254-256.
122. Hasumi K, Kobayashi T, Ata M. Topical idoxuridine for genital condyloma acuminatum. Lancet 1984;i:968.
123. Heino P, Eklund C, Fredriksson-Shanazarian V, Goldman S, Schiller JT, Dillner J. Association of serum immunoglobulin G antibodies against human papillomavirus type 16 capsids with anal epidermoid carcinoma. J Natl Cancer Inst 1995.In press.
124. Heino P, Goldman S, Lagerstedt U, Dillner J. Molecular and serological studies of human papillomavirus among patients with anal epidermoid carcinoma. Int J Cancer 1993;53:377-381.
125. Hellberg D. On some possible etiological factors of cervix and penis cancer with special reference to smoking, sexual behaviour and contraception. Thesis Uppsala University, 1987.
126. Hellberg D, Nilsson S, Haley NJ, Hoffmann D, Wynder E. Smoking and cervical intraepithelial neoplasia: nicotine and cotinine in serum and cervical mucus in smokers and nonsmokers. Am J Obstet Gynecol 1988;158:910-913.
127. Hellberg D, Valentin J, Eklund T, Nilsson S. Penile cancer: Is there an epidemiological role for smoking and sexual behaviour? Br Med J 1987;295:1306-1308.
128. Herrero R, Potischman N, Brinton LA, Reeves WC, Brenes MM, Tenorio F, Britton RCD, Gaitan E. A case-control study of nutrient status and invasive cervical cancer. Am J Epidemiol 1991;134:1335-1346.
129. Higgins GD, Uzelin DM, Phillips GE, Villa LL, Burrell CJ. Differing prevalence of human papillomavirus RNA in penile dysplasias and carcinomas may reflect differing etiologies. Am J Clin Pathol 1992;97:272-278.
130. Hippeläinen M, Syrjänen S, Hippeläinen M, Koskela H, Pulkkinen J, Saarikoski S, Syrjänen K. Prevalence and risk factors of genital human papillomavirus (HPV) infections in healthy males: a study on Finnish conscripts. Sex Transm Dis 1993;20:321-328.
131. Hippeläinen M, Ylikoski M, Saarikoski S, Syrjänen S, Syrjänen K. Genital human papillomavirus lesions of the male sexual partners: the diagnostic accuracy of penoscopy. Genitourin Med 1991;67:291-296.

132. Ho GYF, Burk RD, Fleming I, Klein RS. Risk of genital human papillomavirus infection in women with human immunodeficiency virus-induced immunosuppression. Int J Cancer 1994;56:788-792.
133. Ho L, Tay S-K, Chan S-Y, Bernard H-U. Sequence variants of human papillomavirus type 16 from couples suggest sexual transmission with low infectivity and polyclonality in genital neoplasia. J Infect Dis 1993;168:803-809.
134. Holladay EB, Gerald WL. Viral gene detection in oral neoplasms using the polymerase chain reaction. Am J Clin Pathol 1993;100:36-40.
135. Hori Y, Yamate T. Bowenoid lesions with spontaneous regression. Rinsho Dermatol 1976;18:1067-1072.
136. Hörding U, Junge J, Daugaard S, Lundvall F, Poulsen H, Bock JE. Vulvar squamous cell carcinoma and papillomaviruses: indications for two different etiologies. Gynecol Oncol 1994;52:241-246.
137. Ikenberg H, Gissmann L, Gross G, Grussendorf-Conen EI, zur Hausen H. Human papillomavirus type-16-related DNA in genital Bowen´s disease and in bowenoid papulosis. Int J Cancer 1983;32:563-565.
138. Inman GJ, Cook ID, Lau RKW. Human papillomaviruses, tumour suppressor genes and cervical cancer. Int J STD & AIDS 1993;4:128-134.
139. Jablonska S, Majewski S. Epidermodysplasia verruciformis: Immunological and clinical aspects. Curr Top Microbiol Immunol 1994;186:157-175.
140. Jablonska S, Majewski S, Malejczyk J. HPV infection and immunological responses. In: GPVI Genitoanal Papillomavirus Infection A survey for the clinician (eds: von Krogh, G; Rylander, E) Conpharm AB / KABI/Pharmacia AB, Karlstad, Sweden 1989;289-329.
141. Jalal H, Sanders JH, Prime SS, Scully C, Maitland NJ. Detection of human papillomavirus type 16 DNA in oral squames from normal young adults. J Oral Pathol Med 1992;21:465-470.
142. Jenison SA, Xu XP, Valentine JM, Galloway DA. Characterization of human antibody-receptive epitopes encoded by human papillomavirus types 16 and 18. J Virol 1991;65:1208-1218.
143. Jenison SA, Yu XP, Valentine JM, Koutsky L, Christiansen AE, Beckman AM, Galloway DA. Evidence of prevalent genital-type

human papillomavirus infections in adults and children. J Infect Dis 1990;162:60-69.
144. Jensen SL. Comparison of podophyllin application with simple surgical excision in clearance and recurrence of perianal condylomata acuminata. Lancet 1985;ii:1146-1148.
145. Jochmus-Kudielka I, Schneider A, Braun R, Kimmig R, Koldovsky U, Schneweis KE, Seedorf K, Gissman L. Antibodies against the human paillomavirus type 16 early proteins in human sera: Correlation of anti-E7 reactivity with cervical cancer. J Natl Cancer Inst 1989;81:1698-1704.
146. Jones CJ, Brinton LA, Hamman RF, Stolley PD, Lehman HF, Levine RS. Risk factors for in situ cervical cancer: results from a case-control study. Cancer Res 1990;50:1357-1362.
147. Kashima HK, Shah F, Lyles A, Glackin R, Muhammad N, Turner L, van Zandt S, Whitt S, Shah K. A comparison of risk factors in juvenil-onset and adult-onset recurrent respiratory papillomatosis. Laryngoscope 1992;102:9-13.
148. Kast WM, Brandt RM, Sidney J, Drijfhout JW, Kubo RT, Grey HM, Melief CJ, Sette A. Role of HLA-A motifs in identification of potential CTL epitopes in human papillomavirus type 16 E6 and E7 proteins. J Immunol 1994;152:3904-12.
149. Kataja V, Syrjänen S, Mäntyjärvi R, Ylikoski M, Saarikoski S, Syrjänen K. Prognostic factors in cervical papillomavirus infections. Sex Transm Dis 1992;19:154-160.
150. Kataoka A, Claesson U, Hansson BG, Eriksson M, Lindh E. Human papillomavirus infection of the male diagnosed by Southern-blot hybridization and the polymerase chain reaction: Comparison between urethral samples and penile biopsy samples. J Med Virol 1991;33:159-164.
151. Kienzler JL, Lemoine MT, Orth G, Jibard N, Blanc D, Laurent R, Agache P. Humoral and cell-mediated immunity to human papillomavirus type 1 (HPV-1) in human warts. Br J Dermatol 1983;108:665-672.
152. Kimura S, Hirai A, Harada R, Nagashima M. So-called multicentric pigmented Bowen´s disease. Dermatologica 1978;157:229-237.
153. Kinghorn GR, McMillan A, Mulcahy F, Drake S, Lacey C, Bingham JS. An open, comparative, study of the efficacy of 0.5% podophyllotoxin lotion and 25% podophyllotoxin solution in the

treatment of condylomata acuminata in males and females. Int J STD & AIDS 1993;4:194-199.
154. Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle ELISA detect serum antibodies in a majority of women infected with human papillomavirus type 16. J Natl Cancer Inst 1994;86:494-498.
155. Kirnbauer R, Taub J, Greenstone H, Roden R, Durst M, Gissman L, Lowy DR, Schiller JT. Efficient self-assembly of human papillomavirus type 16 L1 and L1-L2 into virus-like particles. J Virol 1993;67:6929-6936.
156. Kjaer SK, Dahl C, Engholm G, Bock JE, Lynge E, Jensen OM. Case-control study of risk factors for cervical neoplasia in Denmark. II. Role of sexual activity, reproductive factors, and venereal infections. Cancer Causes Control 1992;3:339-348.
157. Kjaer SK, Engholm G, Teisen C, Haugaard BJ, Lynge E, Christensen RB, Möller KA, Jensen H, Poll P, Vestergaard BF, de Villiers E-M, Jensen OM. Risk factors for cervical human papillomavirus and herpes simplex virus infections in Greenland and Denmark: a population-based study. Am J Epidemiol 1990;131:669-682.
158. Kling AR. Genital warts-therapy. Semin Dermatol 1992;11:247-255.
159. Komly CA, Breitburd F, Croissant O, Streeck RE. The L2 open reading frame of human papillomavirus type 1a encodes a minor structural protein carrying type-specific antigens. J Virol 1986;60:813-816.
160. Koss LG, Durfee GR. Unusual patterns of squamous epithelium of the uterine cervix. Ann NY Acad Sci 1956;63:1245-1261.
161. Koss LG, Stewart FW, Foote FW, Jordan MJ, Bader GM, Day E. Some histological aspects of behavour of epidermoid carcinoma in situ and related lesions of the uterine cervix: a long-term prospective study. Cancer 1963;16:1160-1211.
162. Koutsky L, Galloway DA, Holmes KK. Epidemiology of genital human papillomavirus infection. Epidemiol Rev 1988;10:122-163.
163. Koutsky L, Holmes KK, Critchlow CW, Stevens CE, Paavonen J, Beckmann AM, DeRouen TA, Galloway DA, Vernon D, Kiviat N. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N Engl J Med 1992;327:1272-1278.

164. Krauss SJ, Stone KM. Management of genital infection caused by human papillomavirus. Rev Infect Dis (Suppl 6) 1990;12:620-632.
165. Krchnak V, Vagner J, Suchankova A, Krcmar M, Ritterova L, Vonka V. Synthetic peptides derived from E7 region of human papillomavirus type 16 used as antigens in ELISA. J Gen Virol 1990;71:2719-2724.
166. Krebs H-B, Helmkamp BF. Does the treatment of genital condylomata in men decrease the treatment failure rate of cervical dysplasia in the female sexual partner? Obstet Gynecol 1990;76:660-663.
167. Krebs H-B, Helmkamp BF. Treatment failure of genital condylomata acuminata in women: role of the male sexual partner. Am J Obstet Gynecol 1991;165:337-340.
168. Krebs HB. Prophylactic topical 5-fluorouracil following treatment of human papillomavirus associated lesions of the vulva and vagina. Obstet Gynecol 1986;68:837-841.
169. Krebs HB, Schneider A. Human papillomavirus-associated lesions of the penis: colposcopy, cytology and histology. Obstet Gynecol 1987;70:299-304.
170. Köchel HG, Monazahian M, Sievert K, Höhne M, Thomssen C, Teichmann A, Arendt P, Thomssen R. Occurrence of antibodies to L1, L2, E4 and E7 gene products of human papillomavirus types 6b, 16 and 18 among cervical cancer patients and controls. Int J Cancer 1991;48:682-688.
171. Lacey CJN. Genital warts in children. Papillomavirus Report 1991;2:31-33.
172. Lacey CJN. Assessment of exposure to sexually transmitted agents other than human papillomavirus. In: N Munoz, FX Bosch, KV Shah and A Meheus (ed) The epidemiology of cervical cancer and human papillomavirus, IARC, Lyon 1992;93-105.
173. Lassus A. Comparison of podophyllotoxin and podophyllin in treatment of genital warts. Lancet 1987;ii:512-513.
174. Layde PM, Broste SK. Carcinoma of the cervix and smoking. Biomed Pharmacother 1989;43:161-165.
175. Lehtinen M, Dillner J, Luostarinen T, Aromaa A, Hibma M, Paavonen J, Schiller J, Hakama M. Prospective seroepidemiological evidence that both human papillomavirus type 16 and C. Trachomatis infections increase the risk for cervical cancer. 13th

International Papillomavirus Conference, Amsterdam 1994;Abstract 33.
176. Lehtinen M, Leminen A, Kuoppala T, Tiikkainen M, Lehtinen T, Lehtovirta P, Punnonen R, Vestrinen E, Paavonen J. Pre- and posttreatment serum antibody responses to HPV 16 E2 and HSV 2 ICP8 proteins in women with cervical carcinoma. J Med Virol 1992;37:180-186.
177. Lehtinen M, Leminen A, Paavonen J, Lehtovirta P, Hyöty H, Vesterinen E, Dillner J. Serum antibodies to an HPV18 open reading frame E2 derived synthetic peptide predominate in patients with cervical adenocarcinoma. J Clin Pathol 1992;45:494-497.
178. Lehtinen M, Leminen A, Paavonen J, Lehtovirta P, Hyöty H, Vestrinen E, Dillner J. Predominance of serum antibodies to synthetic peptide stemming from HPV 18 open reading frame E2 in cervical adenocarcinoma. J Clin Pathol 1992;45:494-497.
179. Lehtinen M, Niemelä J, Dillner J, Parkkonen P, Nummi T, Liski E, Nieminen P, Reunala T, Paavonen J. Evaluation of serum antibody response to newly identified B-cell epitope in the minor nucleocapsid protein L2 of human papillomavirus type 16. Clin Diagn Virol 1993;1:153-165.
180. Lehtinen M, Parkkonen P, Luoto H. Antipeptide IgA antibodies to a human papillomavirus type 16 E2 derived synthetic peptide predict the natural history of cervical HPV infection. Serono Symposia Publications 1990;78:509-519.
181. Leptak C, Ramon S, Kulke R, Horwitz BH, RieseII DJ, Dotto GP, DiMaio D. Tumorigenic transformation of murine keratinocytes by the E5 genes of bovine papillomavirustype 1 and human papillomavirus type 16. J Virol 1991;65:7078-7083.
182. Ley C, Bauer HM, Reingold A, Schiffman MH, Chambers JC, Tashiro CJ, Manos MM. Determinants of genital human papillomavirus infection in young women. J Natl Cancer Inst 1991;83:997-1003.
183. Li CCH, Shah KV, Seth A, Gilden RV. Identification of the human papillomavirus type 6b L1 open reading frame protein in condylomas and corresponding antibodies in human sera. J Virol 1987;61:2684-2690.
184. Licciardone J, Wilkins JR, Brownson RC, Chang JC. Cigarette smoking and alcohol consumption in the aetiology of uterine cervical cancer. Int J Epidemiol 1989;18:533-537.

185. Lloyd K. Multicentric pigmented Bowen´s disease of the groin. Arch Dermatol 1970;101:48-51.
186. Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster WD, Kurman RJ. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol 1992;79:328-337.
187. Lusky M, Botchan MR. Characterization of the bovine papillomavirus plasmid maintenance sequences. Cell 1984;36:391-401.
188. Lusky M, Botchan MR. Genetic analysis of bovine papillomavirus type 1 trans-acting replication factors. J Virol 1985;53:955-965.
189. Löwhagen G-B, Bolmstedt A, Ryd W, Voog E. The prevalence of "high-risk" HPV types in penile condyloma-like lesions: correlation between HPV type and morphology. Genitourin Med 1993;69:87-90.
190. Maddox P, Szarewski A, Dyson J, Cuzick J. Cytokeratin expression and acetowhite change in cervical epithelium. J Clin Pathol 1994;47:15-17.
191. Maden C, Sherman KJ, Beckmann AM, Hislop TG, Teh C-Z, Ashley RL, Daling JR. History of circumcision, medical conditions, and sexual activity and risk of penile cancer. J Natl Cancer Inst 1993;85:19-24.
192. Majaros M, Devine KD, Parkhill EM. Malignant transformation of benign laryngeal papillomas in children after radiation therapy. Surg Clin North Am 1963;43:1149-1161.
193. Maloney KE, Wiener JS, Walther PJ. Oncogenic human papillomaviruses are rarely associated with squamous cell carcinoma of the bladder: Evaluation by differential polymerase chain reaction. J Urol 1994;154:360-364.
194. Mann VM, Loo de Lao S, Brenes M, Brinton LA, Rawis JA, Green M, Reeves WC, Rawis WE. Occurrence of IgA and IgG antibodies to select peptides representing human papillomavirus type 16 among cervical cancer cases and controls. Cancer Res 1990;50:7815-7819.
195. Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. Use of the polymerase chain reaction amplification for the detection of genital human papillomaviruses. In: Cancer Cells, 7, Molecular diagnostics of human cancer Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1989;209-214.

196. Marchioni M, Penna C, Falani MG, Bracco GL, Bellanti G, Taddei G, Colafranceschi M. Colposcopic features in male partners of women affected by genital condylomatosis. Why a different incidence? Cervix 1988;6:295-301.
197. Martinez I. Relationships of squamous cell carcinoma of the cervix uteri to squamous carcinoma of the penis among Puerto Rican women married to men with penile carcinoma. Cancer 1969;24:777-780.
198. Matlashewski G, Schneider J, Banks L, Jones N, Murray A, Crawford L. Human papillomavirus type 16 DNA cooperates with activated ras in transforming primary cells. EMBO J 1987;6:1741-1746.
199. Mazurkiewicz W, Jablonska S. Comparison between the therapeutic efficacy of 0.5% podophyllotoxin preparations and 20% podophyllin ethanol solution in condylomata acuminata. Z Hautkr 1986;61:1387-1395.
200. Mc Kay M, Frankman O, Horowitz BJ, Lecart C, Michiletti L, Ridley CM, Turner ML, Woodruff JD. Vulvar vestibulitis and vestibular papillomatosis. Report of the ISSVD committee on vulvodynia. J Reprod Med 1991;36:413-415.
201. McCance DJ, Kalache A, Ashdown K, Andrade L, Menezes F, Smith P, Doll R. Human papillomavirus types 16 and 18 in carcinomas of the penis from Brazil. Int J Cancer 1986;37:55-59.
202. McCormick JS. Cervical smears: a questionable practice? Lancet 1989;ii:207-209.
203. McDonnell JM, Mayr AJ, Martin WC. DNA of human papillomavirus type 16 in dysplastic and malignant lesions of the conjunctiva and cornea. N Engl J Med 1989;320:1442-1446.
204. Meisels A, Fortin R, Roy M. Condylomatous lesions of the cervix: II. Cytologic, colposcopic and histopathologic study. Acta Cytol 1977;21:379-390.
205. Melbye M, Coté TR, Kessler L, Gail M, Biggar RJ. AIDS/Cancer working group: High incidence of anal cancer among AIDS patients. Lancet 1994;343:636-639.
206. Melbye M, Rabkin CS, Frisch M, Biggar RJ. Changing patterns of anal cancer incidence in the USA, period 1940-89. Am J Epidemiol 1994;139:772-780.
207. Melkert PWJ, Hopman E, van den Brule AJC, Risse EKJ, van Diest PJ, Bleker OB, Helmerhorst T, Schipper MEI, Meijer CJLM,

Walboomers JMM. Prevalence of HPV in cytomorphologically normal cervical smears, as determined by the polymerase chain reaction, is age-dependent. Int J Cancer 1993;53:919-923.
208. Mendis LN, Best JM, Anatvala JE. Class-specific antibodies (IgG and IgA) to membrane antigens of herpes simplex type-2 infected cells in patients with cervical dysplasia and neoplasia. Int J Cancer 1981;27:669-677.
209. Mitchell H, Medley G, Giles G. Cervical cancer diagnosed after negative results on cervical cytology: perspective in the 1980s. Br Med J 1990;300:1622-1626.
210. Modan B. Screening for cervical cancer - should the routine be challenged? Eur J Cancer 1993;29:2320-2325.
211. Monsonego J, Zerat L, Catalan F, Coscas Y. Genital human papillomavirus infections: correlation of cytological, colposcopic and histological features with viral types in women and their male partners. Int J STD & AIDS 1993;4:13-20.
212. Morison WL. Viral warts, herpes simplex and herpes zoster in patients with secondary immune deficiencies and neoplasms. Br J Dermatol 1975;92:625-630.
213. Morris HH, Gatter KC, Sykes G, Casemore V, Mason DY. Langerhans´ cells in human cervical epithelium: effects of wart virus infection and intraepithelial neoplasia. Br J Obstet Gynecol 1983;90:412-420.
214. Moscicki A-B, Palefsky J, Gonzales J, Schoolnik GK. Human papillomavirus infection in sexually active adolescent females: prevalence and risk factors. Pediatr Res 1990;28:507-513.
215. Moy R, Eliezri YD. Significance of human papillomavirus-induced squamous cell carcinoma to dermatologists. Arch Dermatol 1994;130:235-238.
216. Moy RL, Eliezri YD, Nuovo GJ, Zitelli JA, Bennett RG, Silverstein SJ. Human papillomavirus type 16 DNA in periungual squamous cell carcinomas. JAMA 1989;261:2669-2673.
217. Mueller M, Viscidi RP, Sun Y, Guerrero E, Hill PM, Shah F, Bosch FX, Munoz N, Gissman L, Shah KV. Antibodies to HPV 16 E6 and E7 proteins as markers for HPV 16-associated invasive cervical carcinoma. Virology 1992;187:508-514.
218. Munger K, Phelps WC, Bubb V, Howley PM, Schlegel R. The E6 and E7 genes of the human papillomavirus type 16 together are

necessary and sufficient for transformation of primary human keratinocytes. J Virol 1989;63:4417-4421.
219. Nasiell K, Roger V, Nasiell M. Behaviour of mild cervical dysplasia during long-term follow-up. Obstet Gynecol 1986;67:665-669.
220. Negrini BP, Schiffman MH, Kurman RJ. Oral contraceptive use, human papillomavirus infection, and risk of early cytological abnormalities of the cervix. Cancer Res 1990;50:4670-4675.
221. Noffsinger A, Witte D, Fenoglio-Preiser CM. The relationship of human papillomaviruses to anorectal neoplasia. Cancer 1992;70 (Suppl.):1276-1289.
222. Nuovo GJ, Becker J, Margiotta M, MacConnell P, Comite S, Hochman H. Histological distribution of polymerase chain reaction-amplified human papillomavirus 6 and 11 DNA in penile lesions. Am J Surg Pathol 1992;16:269-275.
223. Nuovo GJ, Hochman HA, Eliezri YD, Lastarria D, Comite SL, Silvers DN. Detection of human papillomavirus DNA in penile lesions histologically negative for condylomata: analysis by in situ hybridization and the polymerase chain reaction. Am J Surg Pathol 1990;14:829-836.
224. Nuovo GJ, Lastarria DA, Smith S, Lerner R, Comite SL, Eliezri YD. Human papillomavirus segregation patterns in genital and non-genital warts in prepubertal children and adults. Am J Clin Pathol 1991;95:27-34.
225. Obalek S, Glinski W, Haftek M. Comparative studies on cell-mediated immunity in patients with different warts. Dermatologica 1980;161:73-83.
226. Obalek S, Jablonska S, Beaudenon S, Walczak L, Orth G. Bowenoid papulosis of the male and female genitalia: Risk of cervical neoplasia. Am Acad Dermatol 1986;14:433-444.
227. Ogilvie M. Serological studies with human papova (wart) virus. J Hyg Camb 1970;68:479-490.
228. Ogunbiyi OA, Scholefield JH, Robertson G, Smith JHF, Sharp F, Rogers K. Anal human papillomavirus infection and squamous neoplasia in patients with invasive vulvar cancer. Obstet Gynecol 1994;83:212-216.
229. Oriel JD. Natural history of genital warts. Br J Vener Dis 1971;47:1-13.

230. Oriel JD. Pathogenisis. In: GPVI Genitoanal Papillomavirus Infection A survey for the clinician (eds: von Krogh, G; Rylander, E) Conpharm AB / KABI/Pharmacia AB, Karlstad, Sweden 1989;ii:9-17.
231. Oriel JD. Sexually transmitted diseases in children: human papillomavirus infection. Genitourin Med 1992;68:80-83.
232. Palefsky JM. Anal papillomavirus infection and anal cancer in HIV-positive individuals: an emerging problem. AIDS 1994;8:283-295
233. Palefsky JM, Holly FA, Gonzales J, Lamborn K, Hollander H. Natural history of anal cytologic abnormalities and papillomavirus infection among homosexual men with Group IV HIV disease. J AIDS 1992;5:1258-1265.
234. Palmer JG, Scholefield JH, Coates PJ, Shepherd NA, Jass JR, Crawford LV, Northover JMA. Anal cancer and human papillomaviruses. Dis Col Rect 1989;32:1016-1022.
235. Papanicolaou GN. Diagnostic value of exfoliated cells. JAMA 1946;131:372-378.
236. Park JS, Namkoong SE, Han SK, Nha DJ, Lee HY, Kim SJ. Comparison of L1 consensus primers with E6 type specific primers for detection of human papillomaviruses in paraffin sections of cervical neoplasia. Journal of Korean Medical Science 1993;8:60-67.
237. Penn I. Tumors of the immunocomprised patient. Ann Rev Med 1988;39:63-73.
238. Peters RK, Thomas D, Hagan DG. Risk factors for invasive cervical cancer among Latinos and non-Latinos in Los Angeles county. J Natl Cancer Inst 1986;77:1063-1077.
239. Petti L, Nilsson L, DiMaio D. Activation of the PDGF receptor by the bovine papillomavirus E5 transforming protein. EMBO J 1991;10:845-855.
240. Pfister H. Human papillomaviruses and genital cancer. Adv Cancer Res 1987;48:113-147.
241. Phil ASD. Epidemiological features of lower genital tract neoplasia. In: GPVI Genitoanal papillomavirus infection A survey for the clinician (eds: von Krogh, G; Rylander, E) Conpharm AB/ KABI/Pharmacia AB, Karlstad, Sweden 1989;Chapter VIII:ii:233-259.
242. Pontén J, Adami H-O, Bergström R, Dillner J, Friberg L-G, Gustafsson L, Miller A, Parkin M, Sparén P, Trichopoulos D.

Strategies for global control of cervical cancer. Int J Cancer 1995.In press.
243. Purdie KJ, Sexton CJ, Proby CM, Glover MT, Williams AT, Stables JN, Leigh IM. Malignant transformation of cutaneous lesions in renal allograft patients: A role for human papillomavirus? Cancer Res 1993;53:5328-5333.
244. Pyrhönen S. Human wart-virus antibodies in patients with genital and skin warts. Acta Dermatovenereologica 1978;58:427-432.
245. Pyrhönen S, Johansson E. Regression of warts. An immunohistological study. Lancet 1975;i:592.
246. Pyrhönen S, Penttinen K. Wart-virus antibodies and the prognosis of wart disease. Lancet 1972;i:1330.
247. Quan MB, Moy RL. The role of human papillomavirus in carcinoma. J Am Acad Dermatol 1991;25:698-705.
248. Rabbett WF. Juvenile laryngeal papillomatosis. The relation of irradiation to malignant degeneration in this disease. Ann Otol Rhinol Laryngol 1965;74:1149-1163.
249. Reeves WC, Rawls JA, Green M, Rawls WE. Antibodies to human papillomavirus type 16 in patients with cervical neoplasia. Lancet 1990;ii:551-552.
250. Reid R, Stanhope CR, Herschman BR, Booth E, Phibbs GD, Smith JP. Genital warts and cervical cancer. I. Evidence of and association between subclinical papillomavirus infection and cervical malignancy. Cancer 1982;50:377-387.
251. Reid R, Stanhope R, Herschman BR. Genital warts and cervical cancer. IV. A colposcopic index for differentiating subclinical papillomaviral infection from cervical intraepithelial neoplasia. J Obstet Gynecol 1984;149:815-823.
252. Reynolds M, Murphy M, Waugh MA, Lacey CJN. An audit of treatment of genital warts: opening the feedback loop. Int J STD & AIDS 1993;4:226-231.
253. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. Am J Obstet Gynecol 1969;105:386-393.
254. Rigorni-Stern. Fatti statistically relativi alle malatti cancerose. Gior Servire Progr Therap 1842;2:507-517.
255. Ringertz N. Tumors of the nose and paranasal sinuses. Acta Otolaryngol Suppl (Stockholm) 1938;27:31-42.

256. Riva JM, Sedlacek TV, Cunnane MF, Mangan CE. Extended carbon dioxide laser vaporization in the treatment of subclinical papillomavirus infection of the lower genital tract. Obstet Gynecol 1989;73:25-30.
257. Rock B, Shah KV, Farmer ER. A morphologic, pathologic, and virologic study of anogenital warts in men. Arch Dermatol 1992;127:495-500.
258. Roitt IM. Essential Immunology. Blackwell scientific publications LTD, London 1991;7th Edition.
259. Roman A, Fife F. Human papillomavirus DNA associated with foreskins of normal newborns. J Infect Dis 1986;153:855-861.
260. Rosemberg SK, Herman G, Elfont E. Sexually transmitted papillomaviral infection in the male. VII. Is cancer of penis sexually transmitted? Urology 1991;37:437-440.
261. Rosenfeld WD, Rose E, Vermund SH, Schreiber K, Burk RD. Follow-up evaluation of cervicovaginal human papillomavirus infection in adolescents. J Pediatr 1992;121:307-311.
262. Rosenfeld WD, Vermund SH, Wentz SJ, Burk RD. High prevalence rate of human papillomavirus infection and association with abnormal Papanicolaou smears in sexually active adolescents. Am J Dis Child 1989;143:1443-1447.
263. Rylander E, Ruusuvaara L, Almströmer MW, Evander M, Wadell G. The absence of vaginal HPV 16 DNA in women who have not experienced sexual intercourse. Obstet Gynecol 1994;83:735-737.
264. Sau P, McMarlin SL, Sperling LC, Katz R. Bowen´s disease of the nail bed and periungual area. Arch Dermatol 1994;130:204-209.
265. Sawchuk WS. Vulvar manifestations of human papillomavirus infection. Dermatol Clin 1992;10:405-414.
266. Sawchuk WS, Weber PJ, Lowey DR, Dzubow LM. Infectious papillomavirus in the vapor of warts treated with carbon dioxide laser or electrocoagulation: detection and protection. J Am Acad Dermatol 1989;21:41-49.
267. Scapier J, Day E, Durfee GR. Intraepithelial carcinoma of the cervix: a cytohistological and clinical study. Cancer 1952;5:315-323.
268. Scheffner M, Munger K, Byrne JC, Howley PM. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc Natl Acad Sci 1991;88:5523-5527.

269. Scheffner M, Romanczuk H, Munger K, Huibregtse JM, Mietz JA, Howley PM. Functions of human papillomavirus proteins. Curr Top Microbiol Immunol 1994;186:83-99.
270. Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB, Scott PR, Sherman ME, Kurman RJ, Wacholder S, Stanton CK, Manos MM. Epidemiological evidence that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993;85:958-963.
271. Schiller JT, Vass WC, Vousden KH, Lowy DR. The E5 open reading frame of bovine papillomavirus type 1 encodes a transforming gene. J Virol 1986;57:1-6.
272. Schneider A. Pathogenesis of genital HPV infection. Genitourin Med 1993;69:165-173.
273. Schneider A, Kirchmayr R, de Villiers E-M, Gissmann L. Subclinical human papillomavirus infections in male sexual partners of female carriers. J Urol 1988;140:1431-1434.
274. Schneider A, Meinhardt G, Kirchmayr R, Schneider V. Prevalence of human papillomavirus genomes in tissues from the lower genital tract as detected by molecular in situ hybridization. Int J Gynecol Pathol 1991;10:1-14.
275. Schoen EJ. The status of circumcision of newborns. N Engl J Med 1990;322:1308-1311.
276. Schoen EJ. The relationship between circumcision and cancer of the penis. CA Cancer J Clin 1991;41:306-309.
277. Scholefield JH, Hickson WGE, Smith JHF, Rogers K, Sharp F. Anal intraepithelial neoplasia: part of a multifocal disease process. Lancet 1992;340:1271-1273.
278. Schultz RE, Miller JW, MacDonald GR, Auman JR, Peterson NR, Ward BE, Crum CP. Clinical and molecular evaluation of acetowhite genital lesions in men. J Urol 1990;143:920-923.
279. Schultz RE, Skelton HG. Value of acetic acid screening for flat genital condylomata in men. J Urol 1988;139:777-779.
280. Seck AC, Faye MA, Critchlow CW, Mbaye AD, Kuypers J, Woto-Gaye G, Langley C, de Benga E, Holmes KK, Kiviat NB. Cervical intraepithelial neoplasia and human papillomavirus infection among Senegalese women seropositive for HIV-1 or HIV-2 or seronegative for HIV. Int J STD & AIDS 1994;5:189-193.

281. Sedlacek TV, Cunnane M, Carpiniello V. Colposcopy in the diagnosis of penile condyloma. Am J Obstet Gynecol 1986;154:494-496.
282. Sedlacek TV, Lindheim S, Eder C, Hasty L, Woodland M, Ludomirsky A, Rando RF. Mechanisms for human papillomavirus transmission at birth. Am J Obstet Gynecol 1989;161:55-59.
283. Sehgal A, Murthy NS, Satyanarayana L, Singh V, Luthra UU. Small family norm and uterine cancer. Acta Obstet Gynecol Scand 1989;68:527-528.
284. Shah K, Howley P. Papillomaviruses. In: "Virology" 2nd edition Eds: BN Fields, DM Knipe; Raven Press 1990;1651-1676.
285. Shah K, Kashima H, Polk BF, Shah F, Abbey H, Abramson A. Rarity of Cesarean section in cases of juvenile-onset respiratory papillomatosis. Obstet Gynecol 1986;68:795-799.
286. Shen C-Y, Ho M-S, Chang S-F, Yen M-S, Ng H-T, Huang E-S, Wu C-W. High rate of concurrent genital infections with human cytomegalovirus and human papillomaviruses in cervical cancer patients. J Infect Dis 1993;168:449-452.
287. Shibutani YF, Schoenberg MP, Carpiniello VL, Malloy TR. Human papillomavirus associated with bladder cancer. Urology 1992;40:15-17.
288. Shroyer KR, Greer Jr RO. Detection of human papillomavirus DNA by in situ DNA hybridization and polymerase chain reaction in premalignant and malignant oral lesions. Oral Surg Oral Med Oral Pathol 1991;71:708-713.
289. Simmons PD, Langlet F, Thin RNT. Cryotherapy versus electrocautery in the treatment of genital warts. Br J Vener Dis 1981;57:273-274.
290. Skinner MS, Sternberg WH, Ichimose H, Collins J. Spontaneous regression of bowenoid atypia of the vulva. Obstet Gynecol 1973;42:40-46.
291. Skrabanek P. Cervical cancer screening. Lancet 1987;ii:1432-1433.
292. Slatterly ML, Abbott Jr TM, Robinson LM, French TK, Jolles C, Gardner JW, West DW. Dietary vitamins A, C and E and selenium as risk factors for cervical cancer. Epidemiology 1990;1:8-15.
293. Slatterly ML, Overall Jr JC, Abbott TM. Sexual activity, contraception, genital infections, and cervical cancer: support for a

sexually transmitted disease hypothesis. Am J Epidemiol 1989;130:248-258.
294. Stenkvist B, Bergström R, Eklund G, Fox CH. Papanicolaou smear screening and cervical cancer. JAMA 1984;252:1423-1426.
295. Stone KM, Becker TM, Hadgu A, Krauss SJ. Treatment of external genital warts: a randomized clinical trial comparing podophyllin, cryotherapy, and electrodessication. Genitourin Med 1990;66:16-19.
296. Strand A, Rylander E, Evander M, Wadell G. Genital human papillomavirus infection among patients attending an STD clinic. Genitourin Med 1993;69:446-449.
297. Strauss MJ, Shaw EW, Bunting H, Melnick JL. "Crystalline" virus-like particles from skin papillomas characterized by intranuclear inclusion bodies. Proc Soc Exp Biol Med 1949;72:46-50.
298. Stremlau A, Gissmann L, Ikenberg H, Stark M, Bannasch P, zur Hausen H. Human papillomavirus type 16 related DNA in an anaplastic carcinoma ot the lung. Cancer 1985;55:1737-1740.
299. Strickler HD, Dillner J, Schiffman MH, Eklund C, Glass AG, Greer C, Scott DR, Sherman ME, Kurman RJ, Wacholder S, Manos M. A seroepidemiologic study of HPV infection and incident cervical squamous intraepithelial lesions. Viral Immunol 1995.In press.
300. Suchankova A, Krchnak V, Vagner J, Hamsikova E, Krcmar M, Ritterova L, Vonka V. Epitope mapping of the human papillomavirus type 16 E4 protein by means of synthetic peptides. J Gen Virol 1992;73:429-432.
301. Suchankova A, Krcmar M, Krchnak V, Hamsikova E, Kanka J, Vagner J, Vonka V. Range of HPV 16 E7 antibodies in cervical cancer patients and healthy subjects. Int J Cancer 1992;51:837-838.
302. Sundberg JP. Papillomavirus infections in animals. In: Papillomaviruses and human disease Eds: Syrjänen, K; Gissmann, L; Koss, LG Springer Verlag, Berlin 1987;40-103.
303. Syrjänen KJ. Histological changes identical to those of condylomatous lesions found in the esophageal squamous cell carcinomas. Arch Geschwulstforsch 1982;4S:283-292.
304. Syrjänen KJ, Syrjänen SM. Human papillomavirus DNA found in bronchial squamous cell carcinomas by in situ DNA hybridization. Lancet 1987;17:168-169.
305. Syrjänen S, Happonen RP, Virolainen E, Siivonen L, Syrjänen K. Detection of human papillomavirus stuctural antigens and DNA

types in inverted papillomas and squamous cell carcinomas of the nasal cavities and paranasal sinuses. Acta Otolaryngol (Stockholm) 1987;104:334-341.
306. Syrjänen S, Partanen P, Mäntyjärvi R, Syrjänen K. Sensitivity of in situ hybridization techniques using biotin- and 35S-labeled human papillomavirus (HPV) DNA probes. J Virol Methods 1988;19:225-238.
307. Syrjänen S, Syrjänen K, Mäntyjärvi R, Collan Y, Kärjä J. Human papillomavirus DNA in squamous cell carcinomas of the larynx demonstrated by in-situ hybridization. J Otorhinolaryngol Relat Spec 1987;49:175-186.
308. Syrjänen SM, Syrjänen KJ, Lamberg MA. Detection of HPV DNA in oral squamous cell lesions using in situ DNA hybridization applied on paraffin sections. Oral Surg 1986;62:660-667.
309. Syrjänen SM, von Krogh G, Syrjänen K. Detection of human papillomavirus DNA in anogenital condylomata in men using in situ hybridization applied to paraffin sections. Genitourin Med 1987;63:32-39.
310. Tagami H, Takigawa M, Ogino A, Imamura S, Ofugi S. Spontaneous regression of plane warts after inflammation. Arch Dermatol 1977;113:1209-1213.
311. Takigawa M, Tagami H, Watanabe S, Ogino A, Imamura S, Ofugi S. Recovery process during regression of plane warts. Arch Dermatol 1977;113:1214-1218.
312. Tay S, Ho TH, Lim-Tan SK. Is genital human papillomavirus infection always sexually transmitted? Aust N Z J Obstet Gynecol 1990;30:240-242.
313. Tay SK, Jenkins D, Maddox P, Singer A. Lymphocyte phenotypes in cervical intraepithelial neoplasia and human papillomavirus infection. Br J Obstet Gynecol 1987;94:16-21.
314. Teresita MA. Vulvoscopy in the diagnosis of genital mycosis. The Cervix LFGT 1994;12:35.
315. Tervahauta AI, Syrjänen SM, Mäntijärvi R, Syrjänen KJ. Detection of p53 protein and Ki-67 proliferation antigen in human papillomavirus (HPV)-positive and HPV-negative cervical lesions by immunohistochemical double-staining. Cytopathology 1994;5:282-293.
316. Thierry F. Proteins involved in the control of HPV transcription. Papillomavirus Report 1993;4:27-32.

317. Thierry F, Yaniv M. The BPV-1 E trans-acting protein can be either an activator or repressor of the HPV-18 regulatory region. EMBO J 1987;6:3391-3397.
318. Thin RN. Meatoscopy: a simple technique to examine the distal anterior urethra in men. Int J STD & AIDS 1992;3:21-23.
319. Thin RN. Meatoscopy: an important technique for assessing meatal warts in men. Int J STD & AIDS 1994;5:18-20.
320. Tindle RW, Frazer IH. Immunology of anogenital papillomavirus infection. Aust NZ J Obstet Gynecol 1990;30:370-375.
321. Toh Y, Kuwano H, Tanaka S, Baba K, Matsuda H, Sugimachi K, Mori R. Detection of human papillomavirus DNA in esophageal carcinoma in Japan by polymerase chain reaction. Cancer 1992;70:2234-2238.
322. Towne JE. Carcinoma of the cervix in nulliparous and celibate women. Am J Obstet Gynecol 1955;69:606-613.
323. van den Brule AJC, Claas HCJ, du Maine M, Melchers WJG, Helmerhorst T, Quint WGV, Lindeman J, Meijer CJLM, Walboomers JMM. Use of anti-contamination primers in polymerase chain reaction for the detection of human papillomavirus genotypes in cervical scrapes and biopsies. J Med Virol 1989;29:20-27.
324. van den Brule AJC, Snijders PJF, Meijer CJLM, Walboomers JMM. PCR-based detection of genital HPV genotypes: An update and future perspectives. Papillomavirus Report 1993;4:95-99.
325. van der Graaf Y, Klinkhamer PJJM, Vooijs GP. Effect of population screening for cancer of the uterine cervix in Nijmegen, The Netherlands. Prevent Med 1986;15:582-590.
326. van Doornum GJJ, Prins M, Juffermans LHJ, Hooykaas C, van den Hoek JAR, Coutinho RA, Quint WGV. Regional distribution and incidence of human papillomavirus infections among heterosexual men and women with multiple sexual partners: a prospective study. Genitourin Med 1994;70:240-246.
327. van Le L, Broekhiuzen FF, Janzer-Steele R, Behar M, Samter T. Acetic acid visualization of the cervix to detect cervical dysplasia. Obstet Gynecol 1993;81:293-295.
328. Van Ranst M, J.B. K, Burk RD. Phylogenetic classification of human papillomaviruses: correlation with clinical manifestations. J Gen Virol 1992;73:2653-2660.

329. Viac J, Chardonnet Y, Chignol MC, Schmidt D. Papilloma viruses, warts, carcinoma and Langerhans´ cells. In Vivo 1993;7:207-212.
330. Viac J, Chomel J-J, Chardonnet Y, Aymard M. Incidence of antibodies to human papillomavirus type 1 in patients with cutaneous and mucosal papillomas. J Med Virol 1990;32:18-21.
331. Viac J, Guérin-Reverchon I, Chardonnet Y, Brémond A. Langerhans cells and epithelial cell modifications in cervical intraepithelial neoplasia: Correlation with human papillomavirus infection. Immunobiology 1990;180:328-338.
332. Viac J, Staquet MJ, Miguet M, Chabanon M, Thivolet J. Specific immunity to human papillomavirus (HPV) in patients with genital warts. Br J Vener Dis 1978;54:172-175.
333. Volpers C, Schirmacher P, Streeck RE, Sapp M. Assembly of the major and the minor capsid protein of human papillomavirus type 33 into virus-like particles and tubular structures in insect cells. Virology 1994;200:504-12.
334. von Krogh G. Warts: immunologic factors of prognostic significance. Int J Dermatol 1979;18:195-204.
335. von Krogh G. Penile condylomata acuminata: an experimental model for evaluation of topical self-treatment with 0.5%-1.0% ethanolic preparations of podophyllotoxin for three days. Sex Transm Dis 1981;8:179-186.
336. von Krogh G. Podophyllotoxin for condylomata acuminata eradication. Clinical and experimental comparative studies on Podophyllum lignans, colchicine and 5-fluoro-uracil. Acta Derm Venereol, Suppl 98 (Thesis) 1981.
337. von Krogh G. Podophyllotoxin in serum: absorption subsequent to three-day repeated applications of a 0.5% ethanolic preparation on condylomata acuminata. Sex Transm Dis 1982;9:26-33.
338. von Krogh G. Condylomata acuminata 1983: An up-dated review. Semin Dermatopathol 1983;2:109-129.
339. von Krogh G. Topical self-treatment of penile warts with 0.5% podophyllotoxin in ethanol for four or five days. Sex Transm Dis 1987;14:135-140.
340. von Krogh G. Clinical relevance and evaluation of genitoanal papillomavirus infection in the male. Semin Dermatol 1992;11:229-240.
341. von Krogh G. Topical treatment of HPV lesions of the external genitalia. The Cervix 1992;10:125-131.

342. von Krogh G, Rylander E. Clinical evaluation. In: GPVI Genitoanal papillomavirus infection A survey for the clinician (eds: von Krogh, G; Rylander, E) Conpharm AB/ KABI/Pharmacia AB, Karlstad, Sweden 1989;ii:71-123.
343. von Krogh G, Rylander E. Therapeutic possibilities. In: GPVI Genitoanal Papillomavirus Infection A survey for the clinician (eds: von Krogh, G; Rylander, E) Conpharm AB / KABI/Pharmacia AB, Karlstad, Sweden 1989;125-177.
344. von Krogh G, Syrjänen SM, Syrjänen KJ. Advantage of human papillomavirus typing in the clinical evaluation of genitoanal warts. J Amer Acad Dermatol 1988;18:495-503.
345. von Krogh G, Szpak E, Andersson M, Bergelin I. Self-treatment using 0.25%-0.5% podophyllotoxin ethanol solutions against penile condylomata acuminata: a placebo-controlled comparative study. Genitourin Med 1994;70:105-109.
346. Vonka V, Kanka J, Hirsch I, Zavadova H, Krcmar M, Suchankova A, Rezakova D, Broucek J, Press M, Domorazkova E, Svoboda B, Havrankova AO. Prospective study on the relationship between cervical neoplasia and herpes simplex type-2 virus. II. Herpes simplex type-2 antibody presence in sera taken at enrolment. Int J Cancer 1984;33:61-66.
347. Voog E, Löwhagen G-B. Follow-up of men with genital papillomavirus infection. Acta Derm Venereol 1992;72:185-186.
348. Vousden KH. Human papillomaviruses and cervical carcinoma. Cancer Cells 1989;1:43-50.
349. Watanable S, Kanda T, Yoshiike K. Human papillomavirus type 16 transformation of primary human embryonic fibroblasts requires expression of open reading frames E6 and E7. J Virol 1989;63:965-969.
350. Waugh M. Dermato-venereology: An historical perspective. J Eur Acad Dermatol Venereol 1994;3:551-554.
351. Wells M. Human papillomaviruses and anal neoplasia. Papillomavirus Report 1990;1:1-2.
352. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 1990;248:76-79.
353. Wheeler CM, Parmenter CA, Hunt WC, Becker TM, Greer CE, Hildesheim A, Manos MM. Determinants of genital human papillomavirus infection among cytologically normal women

attending the university of New Mexico student health center. Sex Transm Dis 1993;20:286-289.
354. Wisniewski PM, Warhol MJ, Rando RF, Sedlacek TV, Kemp JE, Fisher JC. Studies on the transmission of viral disease via the CO2 laser plume and ejecta. J Reprod Med 1990;35:1117-1123.
355. Wrede D, Tidy JA, Crook T, Lane D, Vousden KH. Expression of RB and p53 proteins in HPV-positive and HPV-negative cervical carcinoma cell lines. Mol Carcinogenesis 1991;4:171-175.
356. zur Hausen H. The role of papillomaviruses in anogenital cancer. Scand J Infect Dis, Suppl 69 1990;107-111.