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Pathogenesis and treatment of human genital papillomavirus infections: a review

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Summary

HPV infection of the genital tract is common and anogenital warts or condyloma acuminatum is increasing rapidly in incidence. In addition, certain HPV types are closely associated with genital tract malignancies. Although recent advances in molecular biology have led to an increased understanding of the organization and functions of the papillomavirus genome, the pathogenesis of HPV infections and host responses to these diseases remain poorly understood. Treatment of anogenital warts is difficult and no completely satisfactory treatment modality is currently available. Comparatively few therapeutic modalities have been thoroughly evaluated, although recent studies of intralesionally and parenterally administered interferons have demonstrated beneficial effects of interferon compared to placebo. Additional studies of treatment for condyloma acuminatum are needed and should include the use of biologic response modifiers such as interferons, as well as antiviral drugs, with or without conventional methods of local therapy.

HPV infection; Pathogenesis

Introduction

Person-to-person transmission experiments using cell-free extracts from infected tissue suggested in 1907 that viruses were the etiologic agents of warts (Ciuffo, 1907). Other early studies examined the biological behavior of the cottontail rabbit papillomavirus (CRPV) (Shope, 1937). These investigations demonstrated that

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CRPV infects the epithelium of wild rabbits and produces proliferative lesions productive of large numbers of infectious virions. Infection of domestic rabbits, on the other hand, produces similar proliferative lesions but usually very few virus particles. Lesions in domestic rabbits were shown to undergo malignant degeneration with high frequency, establishing CRPV as the earliest identified DNA tumor virus. Electronmicroscopic studies demonstrated later that some types of human warts similarly contain large numbers of viral particles while other types show very low productivity (Pfister, 1984). Despite these interesting early observations, papillomaviruses have not been studied using standard virologic techniques, because they have not been propagated successfully in tissue culture. This inability to grow these viruses in vitro has greatly impaired our understanding of the biology of these agents. For example, as recently as 15 years ago it was generally thought that all human warts were caused by a single type of human papillomavirus (HPV).

Despite the lack of a suitable tissue culture system, recent developments have dramatically increased our ability to perform studies of HPV. Advances in molecular biology have made it possible to clone viral DNA from tissue, identify multiple virus types, and determine the organization and many functions of the papillomavirus genome (Broker, 1987).

The viruses

Papillomavirus and *Polyomavirus* are the two genera that comprise the family Papovaviridae (Shah, 1985). Papillomaviruses are nonenveloped with icosahedral capsids approximately 55 nm in diameter, and circular double-stranded DNA genomes containing approximately 7900 base pairs. Based primarily on studies of bovine papillomavirus type1 (BPV1)-transformed murine cell lines (Dvoretzky et al., 1980), the genome has been functionally divided into three domains: an early (E) region containing 5–7 open reading frames (ORFs) encoding transformation, replication and regulation functions, a late (L) region containing 2 ORFs encoding the major and minor viral capsid proteins, and a long control region (LCR) containing transcription regulatory sequences and the origin of replication (Broker, 1987). At least 12 papillomavirus types of both human and animal origin have been fully sequenced and the genomic organization has been shown to be remarkably conserved. In contrast to the polyoma group, for which early and late proteins are encoded by opposite DNA strands, the papillomavirus open reading frames are all contained in the same strand. This markedly different organization suggests that there is no close evolutionary relationship between the two genera of the Papovaviridae.

At present, 57 different HPV types have been cloned (personal communication, Ethel-Michele de Villiers, HPV Reference Center, Heidelberg, F.R.G.). Serotyping cannot be performed because few type-specific antigens are available. Thus, types are differentiated on the basis of DNA homology. DNA of a unique HPV type must cross-hybridize less than 50% with DNA of other classified HPV types.

HPV subtypes have high percentages of DNA homology but often vary with respect to sites of cleavage by restriction endonucleases.

Papillomaviruses are species specific, and most types show definite tropisms for specific anatomical sites. HPV-1 for example, causes plantar warts while HPV-2 causes common warts, the familiar dome-shaped lesions usually found on the hands. Most studies of genital lesions have demonstrated that HPV types 6 and 11 are associated with lower degrees of dysplasia and with most benign cervical and genital condylomata, whereas types 16 and 18 are associated with more advanced genital tract neoplasias (Armstrong et al., 1988; Campion et al., 1986).

Pathogenesis and immune responses

Although the pathogenesis of HPV infections is incompletely understood, it is generally believed that infection begins with entry of virus into the basal cells of the epithelium. Viral DNA has been detected in the nuclei of these cells (Stoler and Broker, 1986). It is likely that papillomavirus DNA replicates in synchrony with cellular DNA and is partitioned between dividing cells. Productive infection requires differentiation of epithelial cells, and viral structural proteins are expressed only in the fully differentiated superficial epithelial layers. Complete virus particles are assembled in the nuclei of these cells, and infectious virions are presumably released when dead keratinocytes are shed. Viral replication is associated with proliferation of all layers of the epithelium, except the basal layer, producing acanthosis, parakeratosis, and hyperkeratosis. Of note, however, is the observation that not all cells in a wart contain detectable viral DNA or RNA (Stoler and Broker, 1986). It is also important to recognize that HPV DNA may be present in grossly and histologically normal appearing tissues (Ferenczy et al., 1985; Steinberg et al., 1988).

One recently developed animal model of HPV infection may help to define the relationships between viral replication and the formation of characteristic HPV-induced lesions (Kreider et al., 1987). In this system, human foreskin is inoculated with a condylomatous extract, and implanted under the renal capsule of athymic nude mice. Three to four months later, condylomatous cysts develop which contain gram quantities of desquamated cells. Histological examination of the walls of these cysts demonstrates epithelial changes characteristic of HPV infection. Significant quantities of intact HPV-11 virions can be purified from these lesions. In addition to providing a source of virus particles, this system can be used in studies of pathogenesis (Brown et al., 1988).

The mechanisms of host defense to HPV infection are poorly understood. However, several clinical observations have indicated that an intact immune system is important in protection from, and resolution of, clinically apparent HPV infection. Patients with both primary and acquired immunodeficiency are susceptible to frequent and severe HPV disease (Kirchner, 1986). Most recently, patients with human immunodeficiency virus (HIV) infection have been noted to suffer frequently from HPV disease (Matis et al., 1987). Despite these observations, most

patients with HPV disease do not appear to have any consistent immune defect.

Studies of humoral and cellular immunity to papillomaviruses have been reviewed recently (Kirchner, 1986). Most of these studies are difficult to interpret because poorly characterized preparations were used as antigens. More recently, studies have been conducted in which HPV antigens have been produced using DNA recombinant techniques, and employed to detect antibodies in human sera. In one such study, no antibodies were detected in human sera, when betagalactosidase fusion proteins derived from L1 and L2 of HPV-6b were employed as antigens in a Western blot assay (Strike et al., 1989). Other investigators found antibodies in sera from patients attending a sexually transmitted diseases clinic, which were directed against a TrpE fusion protein containing 67% of the major capsid protein of HPV-6b (Jenison, et al., 1988). Appropriately conducted epidemiologic studies will need to be conducted in association with laboratory investigations in order to determine appropriate type-specific antigens to be employed in serological studies. These antigens will also be needed to conduct rigorous evaluations of cell-mediated responses to HPV infection.

Anogenital warts

Natural history

Data from several sources indicate that the incidence of anogenital warts is rapidly increasing (Becker et al., 1985; Chuang et al., 1984). This common sexually transmitted disease has an incubation period of 1–8 months and is transmitted to approximately 2/3 of sexual contacts of infected patients (Goldsmidt and Klingman, 1958; Barrett et al., 1954; Oriel, 1971). The infectivity of lesions is thought to be inversely related to their duration (Oriel, 1971). With the exception of observations of placebo recipients in recently conducted clinical trials, little systematically collected information is available concerning the natural history of these diseases.

In men the most frequent site of involvement is the penis. The urethral meatus may also be involved and such lesions may be associated with more proximal disease. Perianal warts occur commonly among homosexual/bisexual men but are observed among heterosexual men and frequently among women as well. The vulva is the most common site of infection in women, although vaginal and cervical infections also occur frequently. It is important to note that cervical and vaginal HPV infections may be present in the absence of external lesions. Morphology of anogenital warts does not appear to be related to natural history of disease or response to therapy. Anogenital warts represent an often troublesome cosmetic problem. In addition, they may bleed occasionally and cause local discomfort. Complications of condyloma acuminatum include rapid enlargement of lesions during pregnancy producing obstruction of the birth canal, and possibly rare transformation into squamous cell carcinomas, including verrucous carcinoma (Shafeek et al., 1979). HPV disease of the female genital tract is also thought by many investigators to represent the reservoir of infection for most cases of recurrent respiratory papillomatosis (Mounts and Shah, 1984).

Relationship to cancer

The vast majority of warts are benign tumors. Common, flat and plantar warts seldom undergo malignant degeneration. Papillomaviruses have been most clearly related to cancers in rabbits and in the rare human genetic disorder epidermodysplasia verruciformis. Patients with this condition develop infections with multiple human papillomavirus types but only the acquisition of certain types (e.g. types 5,8 and 14) is associated with malignant changes and then in as many as 30% of cases (Grussendorf-Conen, 1987).

Although condyloma acuminatum caused by HPV types 6 and 11 is almost always a benign disease, other HPV types are intimately associated with genital tract malignancies, particularly cervical intraepithelial neoplasias. Although a causal role has not been established (Munoz et al., 1988), a significant body of circumstantial evidence supports the hypothesis that some HPV infections do contribute to the pathogenesis of cervical cancer (Gissmann et al., 1987). As many as 95% of cervical carcinomas contain HPV DNA, particularly of types 16 and 18. Many cell lines, particularly those derived from cervical carcinoma biopsies, contain these same viral nucleic acids. When present in cancers and cell lines, HPV DNA is found to be integrated into the host cell genome, rather than located episomally as it is in benign tumors. Integration of HPV DNA has been shown to occur at specific sites in the HPV genome, although insertion into host cell chromosomes appears to occur at random locations. Integrated papillomavirus DNA is transcriptionally active and is preserved when malignant cells are cultivated in vitro. Several open reading frame products from the early region have been shown to transform rodent cells in tissue culture. A primary human cell line transformed with HPV 16 DNA has been developed (Pirisi et al., 1987). Epidemiological studies suggest that a long latency period between infection with papillomavirus and induction of malignancy would be necessary to account for the differing age incidence of these conditions. It is likely that additional cofactors such as local perturbations of the immune system or interaction with other infectious agents or tumor promoters may be required for oncogenesis.

Diagnosis

Diagnosis of HPV infection is usually made based on the typical appearance of exophytic lesions. However, gross physical examination of visible anogenital lesions is incorrect approximately 10% of the time when compared to histologic examination of biopsy specimens. Molluscum contagiosum may be confused with anogenital warts, and HPV infection of the uterine cervix often produces atypical flat warts. Application of dilute solutions of acetic acid, followed by colposcopy, increases the sensitivity of physical examination of HPV-induced lesions in both men and women (Barrasso et al., 1987). HPV lesions identified by this method have an 'aceto-white' appearance and frequently contain HPV DNA when biopsy specimens are processed appropriately. All atypical genital tract lesions should be biopsied. In addition to demonstrating characteristic histologic features of HPV

infection, biopsy specimens can be examined for the presence of HPV antigens using immunocytochemical techniques, and for the presence of HPV nucleic acids by filter, in situ, or Southern hybridization.

Treatment

Because this sexually transmitted disease is rapidly increasing in frequency and is intimately associated with genital tract neoplasias, it is generally agreed that condyloma acuminatum should be treated. However, completely safe and effective modes of therapy are not available, although a variety of different treatment modalities are currently available (Table 1). Podophyllin, a resin extract from the rhizomes *Podophyllum peltatum* or *P. emodi*, is a principal mode of treatment. Despite initial reports of almost universal success, recent studies have indicated that the rates of complete lesion regression using this mode of therapy are approximately 30%. Podophyllotoxin is a purified preparation of the most active ingredient of podophyllin, and appears to have similar rates of efficacy. Application of both preparations is associated with local burning and occasional ulceration. Rarely, teratogenecity and systemic side effects have also been observed. Cryotherapy, usually utilizing liquid nitrogen spray, is, along with podophyllin, one of the two regimens currently recommended by the Centers for Disease Control for the treatment of external anogenital warts (Centers for Disease Control, 1985). Generally regarded as a safe treatment, cryotherapy may also be more effective than podophyllin (Bashi, 1985).

Several different factors have made it difficult to design, conduct, and interpret results of clinical trials of treatments for condyloma acuminatum. For example, there is a lack of adequate natural history data, and several clinical and virologic measurements of disease which may be of potential importance have not been taken into account in most clinical trials. Thus, virus type, immune status of patients, duration of disease prior to study entry, as well as sex and sexual preference of patients may be important in the natural history of condyloma acuminatum. These factors should be considered in design and execution of appropriate clinical trials.

TABLE 1

Treatment modalities for condyloma acuminatum

1. Podophyllin/podophyllotoxin
2. Cryotherapy
3. 5-fluorouracil
4. Tri- and bi-chloracetic acids
5. Surgery
6. Electrosurgery
7. Laser
8. Infrared coagulation
9. Dinitrochlorobenzene
10. Interferon

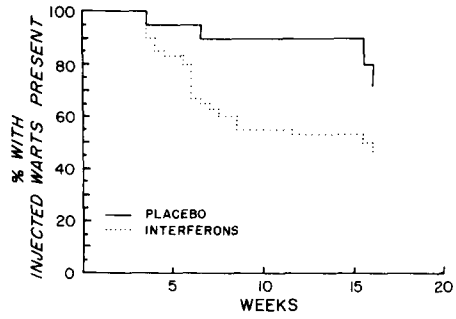

Fig. 1. Complete resolution of injected warts. The proportions of patients whose injected warts were present at the indicated time point are plotted against weeks of observation after study entry. All interferon recipients are combined into one group and compared to placebo. Interferon recipients are represented by the dotted line, and placebo recipients by the solid line. Differences in complete resolution of injected warts between the two groups are highly significant ($P < 0.01$, by log rank test).

Although serial biopsies can be obtained to estimate amounts of viral nucleic acids and antigens in lesions, the only practical, objective measurement of disease which can currently be measured is size and number of lesions. Serial biopsies are difficult to obtain and are subject to sampling error.

As a result of anecdotal observations and small open studies, recent investigations have been conducted to evaluate the potential efficacy of interferons in the treatment of these infections. Four placebo-controlled trials of alpha interferons administered intralesionally in the treatment of condyloma acuminatum have been reported, and have demonstrated similar results (Eron et al., 1986; Friedman-Kien et al., 1988; Vance JC et al., 1986; Reichman et al., 1988b). In addition, one of these studies also evaluated a beta interferon preparation (Reichman et al., 1988b). In this latter study, the clinical diagnosis of condyloma acuminatum was confirmed by histologic examination of biopsy specimens prior to study entry. Subjects were then randomized to receive either 1×10^6 units of an interferon preparation or placebo, which was injected intralesionally three times per week for four weeks. In this study, only one lesion per patient was injected, and interferon preparations which were employed included alpha-n1, alpha-2b, and beta interferons. A total of 76 patients were evaluated. Numbers of patients were not significantly different among the four treatment arms, and most patients were white, heterosexual men

TABLE 2

Frequencies of complete resolution of injected warts after 16 weeks of study

Study medication	Frequencies of resolution (%)
Alpha-n1 interferon	6/15 (40)
Beta interferon	10/20 (50)
Alpha-2b interferon	11/23 (48)
All interferons	27/58 (47)
Placebo	4/18 (22)

in their late 20s. All had received previous conventional therapy and had had disease for approximately 2 years prior to study enrollment. When the interferon groups were combined and compared to placebo, a highly significant difference in rates of complete resolution was seen in the combined interferon group as compared to the placebo group (Fig. 1). As outlined in Table 2, approximately 50% of interferon recipients experienced complete resolution of injected lesions at 16 weeks of study as compared to approximately 20% of placebo recipients. No significant differences in rates of complete lesions resolution were noted among recipients of the three different interferon preparations. Of interest, marked differences from baseline were noted at certain time points between areas of interferon-injected and placebo-injected warts that ultimately did not completely resolve (Fig. 2). At 4, 6, and 8 weeks after enrollment, mean reductions in lesion areas of injected warts were greater in the combined interferon group than in the placebo group. This difference disappeared after the tenth week of observation, suggesting that more prolonged therapy may have been more effective.

In a subset of 43 of these patients, in situ and Southern hybridization techniques were employed to detect virus nucleic acids in tissue. In addition, immunocytochemical methods were employed to detect papillomavirus antigens. Using these methods, response to interferon therapy could be more accurately predicted (Reichman et al., in press). Patients whose lesions contained detectable HPV nucleic acids or papillomavirus antigens, or in which koilocytes were observed, were more likely to respond to interferon treatment than patients whose biopsies did not fulfill these criteria.

In a recently completed trial, this same group of investigators compared three different alpha interferon preparations administered parenterally in the treatment of condyloma acuminatum (Reichman et al., 1988a). In this trial, 178 patients were randomized to receive either 2×10^6 units/m^2 of an interferon or placebo, administered subcutaneously three times per week for four weeks. In contrast to the in-

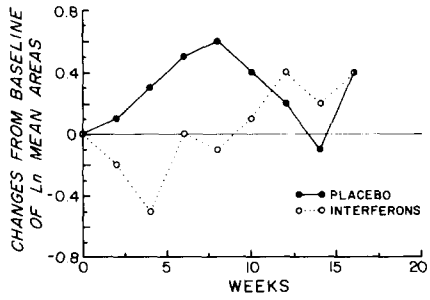

Fig. 2. Changes from baseline in areas of injected warts which did not resolve completely. Areas were expressed as the natural logarithm of mean areas, and changes from baseline are plotted against weeks of observation after study entry. All three interferon groups were combined and compared with the placebo group. Significant differences between interferon and placebo groups were noted at 4, 6 and 8 weeks after study enrollment ($P < 0.05$ for each comparison). No significant differences between the two groups were noted at other time points.

tralesional study, most patients who participated in the parenteral study had received no previous therapy and had had disease for a median of only 8 months. Most patients were white, heterosexual men. When the three interferon groups were combined and compared to the placebo group, no significant difference in rates of complete lesion resolution were observed. However, more interferon recipients than placebo recipients did experience both a 50% and a 25% decrease in total lesion area. Statistically significant differences among the different interferon groups were not observed. Of interest, women experienced rates of complete lesion resolution, independent of treatment, more frequently than did men. As would be expected with these moderate doses of interferon, most interferon recipients experienced typical influenza-like side effects, including headache, fever, chills, myalgias, and malaise. In addition, neutropenia and thrombocytopenia compared to baseline values were observed more frequently among interferon recipients than among placebo recipients. However, no study participant withdrew from the study because of side effects, and dosage adjustments were not required for either side effects or changes in laboratory tests.

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