Immunoperoxidase Demonstration of Human Papilloma Virus (HPV) in Dysplastic Lesions of the Uterine Cervix

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Summary. Routinely processed sections of 47 unselected cervical dysplasias and in situ carcinomas (both condylomatous, CO, and noncondylomatous, NCO) were subjected to staining with indirect immunoperoxidase-PAP technique using anti-human papilloma virus (anti-HPV) immune serum to demonstrate HPV antigens in these lesions. Anti-HPV was raised in guinea pigs immunized with highly purified virions from a pool of human skin wart tissues. Positive reactivity for HPV (brown intranuclear precipitates) was disclosed in 72% of the CO dysplasias and in only one case (5.6%) of the NCO group. The frequency of HPV-positive lesions was inversely related to the degree of epithelial dysplasia, being consistent with the disappearance of ultrastructurally detectable virus particles in lesions turned malignant. HPV-containing cells were characteristic koilocytes or dyskeratotic superficial cells. The present technique provides definite evidence for the HPV etiology of the flat, inverted, and papillomatous condylomas, and seems to offer an advantage as a diagnostic tool of these lesions. When applied to malignant tumors in the near future, the method will undoubtedly bring more light on the suggested role of HPV in the etiology of human squamous cell carcinomas of the uterine cervix and other sites as well.

Key words: Cervical dysplasias – Condylomas – Anti-HPV immune serum – Indirect immunoperoxidase technique – Human squamous cell carcinogenesis

Introduction

Genital warts (condylomata acuminata) caused by human papilloma virus (HPV) [1, 4, 5, 15, 23, 37] are known to manifest themselves as three distinct morphological entities named flat, inverted, and papillomatous condylomas [15–17, 20, 26–31]. As shown in electron microscopy, all these lesions contain HPV particles, albeit in very small numbers rendering their ultrastructural demonstration troublesome [1, 3–5, 8, 10, 13, 14–16, 19].

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Condylomas of the uterine cervix have been subjected to intense study because of the reports on their frequent association with epithelial dysplasias and even invasive carcinomas of the cervix [2-4, 14-17, 20, 26-33], thus suggesting that HPV could be engaged in cervical carcinogenesis [2, 3, 8, 9, 11, 14, 16, 17, 20, 23, 24, 26-33, 36, 37]. Attempts to find HPV particles in such lesions after their malignant conversion have failed, however [8, 9, 24, 36], and other measures to gain evidence for this suggested HPV-cancer association have to be searched for. Recently, an immunoperoxidase technique was successfully applied to cervical condylomatous atypias to demonstrate HPV antigens in the lesions [12, 18, 25, 35]. Our preliminary results in selected cervical condylomas confirmed the applicability of such a technique [34], which in the present work was applied on an unselected series of cervical dysplasias (both condylomatous and non-condylomatous ones included) to assess the presence of HPV antigens in routinely processed biopsy specimens.

**Material and Methods**

The present series consists of colposcopy or conization biopsy specimens from 47 consecutive women diagnosed as dysplasias or in situ carcinomas at Kuopio University Central Hospital, Kuopio, Finland, during 1978-1980. All specimens were fixed in 10% neutral formalin, embedded in paraffin, and stained with hematoxylin-eosin (HE) for diagnostic purposes.

All the lesions were re-evaluated and classified as either condylomatous (CO) or non-condylomatous (NCO) using the morphological criteria recently detailed elsewhere [15-17, 20, 26-31, 33]. The CO changes were further classified as flat, inverted, or papillomatous types, as described previously. The degree of epithelial dysplasia was assessed according to the criteria in common use.

For the demonstration of HPV antigens, an indirect immunoperoxidase-PAP technique applied on paraffin sections was used. The technical details were given recently [34], and will be outlined here only briefly. The deparaffinized 5-μm sections were sequentially incubated with normal goat serum, guinea pig anti-HPV serum, rabbit anti-guinea pig whole serum, goat anti-rabbit whole serum, and rabbit PAP (peroxidase-antiperoxidase soluble complex). Negative controls were parallely processed, and each of the antisera were left out in turn to ensure a fourfold negative control. As positive controls, ten common warts were stained, showing definite positive reaction for HPV in many cells.

The preparation and specificity testing of the primary antiserum used, i.e., guinea pig anti-HPV have been described previously [21, 22]. As an immunizing antigen, whole (native) papilloma virus particles purified from pooled wart tissue collected from more than 20 individuals were used [21, 22]. Such a pool contains warts from different regions of the body, including common warts, plantar warts, anal warts, and genital warts. As determined by complement fixation and immunodiffusion tests, the antisera elaborated exhibited HPV antibody titers of 1:128 and 1:256, respectively, and no precipitation lines were found when tested against the normal skin antigens purified as the viral antigens [21, 22].

**Fig. 1.** A medium power view on a condylomatous lesions stained for HPV. Distinct positive reactions (dark nuclei) are present within the nuclei of the koilocytotic cells in the superficial (S) layers of the epithelium. The cells in the deep (D) layers remain entirely negative in this procedure. (Indirect immunoperoxidase-PAP for HPV, counterstained with hematoxylin, × 250)

**Fig. 2.** Another condylomatous dysplasia with higher power. The majority of the koiocytes (K) stain positively with anti-HPV, as do some of the superficial dyskeratotic cells (arrow). Again, the cells deeper in the epithelium do not contain HPV. (Indirect immunoperoxidase-PAP for HPV, counterstained with hematoxylin, × 375)