Verrucae: Virus Structure, Localization of Antigens, and Comparison with the Shope Papilloma

Wilbur Fiske Noyes

Division of Experimental Pathology, Sloan-Kettering Institute for Cancer Research and Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York, New York

SUMMARY

The fine structure of the human wart virus is compared with that of the Shope rabbit papilloma virus. The distribution of the viral antigens within the human wart, as determined by immunofluorescence, is described and compared with the distribution of the viral antigens in the rabbit papilloma. The distribution of viral particles in the two lesions, as determined by electron microscopy, is also compared.

INTRODUCTION

Verrucae or human warts are circumscribed, benign, contagious, virus-induced epithelial growths of the skin and adjacent mucous membrane (23). The principal pathologic condition is in the epidermis, where there is a varying hyperplasia of the germinal cell, granular cell, or keratin layers, depending on the type and location of the wart (23). The viral etiology of verruca has been known since 1907, when Ciuffo (7) transmitted the lesion by a cell-free filtrate. It is evident that one virus or closely related strains of virus cause several different morphologic types of warts, depending on the local tissue differences, since inoculation of material from one type of wart can give rise to those of another type (10, 22). Experimental inoculation of the virus into human volunteers results in the development of typical verrucae at the inoculation site in from 1 to 8 months (5, 9, 24). With one exception, verrucae have not been transmitted to any experimental animal. It was reported by Ullman in 1923 (22) and confirmed by Ishikawa in 1936 (10) that laryngeal papillomas were transmitted to dogs by injecting papilloma extracts into the vaginal mucosa. Reports of the replication of the virus in tissue culture (12, 18) have not been confirmed. The human wart virus has been recognized morphologically since 1949 when Strauss et al. (21) described particles in shadowed preparations of wart extracts. In 1953 Bunting (6) demonstrated arrays of particles within the nuclei using the then recently developed thin-sectioning technic.

MATERIALS AND METHODS

Density gradient purification of the human wart virus and acid for examination in the electron microscope have been previously described (13). Rabbit antiserum was prepared against the human wart virus by multiple injections of highly purified wart virus mixed with complete Freund's adjuvant.

Preparation of the fluorescein-conjugated antibody, absorption of the conjugate, and methods of staining have been previously described (14).

RESULTS AND DISCUSSION

Structure of the Virus

The ultrastructure of the human wart virus has been described by Williams et al. (25), Berrera-Oro et al. (2), Crawford and Crawford (8), and Noyes (13). Fig. 1 is an electron micrograph, negatively stained with phosphotungstate, of the human wart virus purified by density gradient centrifugation. Complete viral particles are present, together with several abnormal, elongated forms of the virus. The diameter of the normal particles of the human wart virus averages about 57 millimicrons. Williams et al. (25) found the wart virus particles to average about 55 millimicrons. It was determined by Crawford and Crawford (8) and Williams et al. (25) from counting aggregates of capsomeres and from examination of high resolution micrographs of the virus that the shell of the virus particle is composed of 42 capsomeres. A number of the particles in Fig. 1 have been penetrated by the stain which indicates that they are protein shells devoid of DNA. The abnormal elongated forms of the wart virus have a shell composed of normal capsomeres. These abnormal forms are probably due to errors in synthesis in some of the cells producing the virus. It is interesting to compare the morphology of the human wart virus with that of the Shope rabbit papilloma virus, as the lesion is very similar in both hosts. In man, however, the growth remains benign, while in the rabbit it frequently progresses to carcinoma (19). The fine structure of the Shope rabbit papilloma virus has been described by Breedis et al. (4) and by Noyes (16). Fig. 2 is an electron micrograph of a density gradient purified preparation of the papilloma virus negatively stained with phosphotungstate. Note the close resemblance to the human wart virus. The Shope papilloma virus is slightly larger in size than the human wart virus. The average diameter of the particles in Fig. 2 is about 61 millimicrons. The shell of the Shope virus apparently contains more capsomeres than the verruca virus, as Breedis et al. (4) have esti-
mated the number at 60–70. In Fig. 2 note the elongated forms of the Shope virus, which are very similar to those found in human wart preparations.

**Location of Virus Particles and Antigens within the Tumor**

In the Shope rabbit papilloma, immunofluorescent investigations by Noyes and Mellors (17) have shown that the viral antigens are not detectable in proliferating cells in the germinallayer, but are only present in the nuclei of cells in the granular layers (stratum granulosum) and in the keratinized layers (stratum corneum) where the cells are undergoing degeneration and necrosis. It has been shown by Noyes (15)that the infective Shope virus is detectable only in the stratum granulosum and stratum corneum. Electron microscopy has confirmed these findings (20). No viral particles could be detected in the germinall cell layers. Particles were detected only in the nucleoli of cells in the stratum granulosum and as aggregates in the keratin of the stratum corneum.

The cellular distribution of the viral antigens in verrucae was found to be identical with the distribution of the Shope viral antigens in the rabbit papilloma. To determine the location of the wart viral antigens, antiserum prepared in rabbits against purified human wart virus was conjugated with fluorescein. This reagent was used to stain frozen sections of human warts and Fig. 3, a fluorescence photomicrograph, illustrates the typical result. Localization of the viral antigen occurs only in nuclei of cells in the stratum granulosum and in keratin in the stratum corneum. The stratum corneum of the section is oriented at the top of Fig. 3. No viral antigens are detectable in the cells of the germinall layers. The specificity of the staining was determined by blocking reactions with unlabeled immune serum. Electron microscopic studies of thin sections of human warts by Almeida et al. (1) have demonstrated that the viral particles cannot be located in the germinall cell layers and are present only in nucleoli and nuclei of cells in the granular layers and as aggregates of virus embedded in keratin in the cornified layers.

Verrucae frequently regress spontaneously, but this is probably not due to an increase in antibody content of the serum, since none could be demonstrated in patients with regressing lesions (3). Complement fixation has been demonstrated between extracts of verrucae and the serum of individuals with warts (11). However, no attempt has been made to determine if this is due to viral antigens or to tumor-specific antigens. This should be a fruitful area for future study.

**REFERENCES**

Fig. 1. An electron micrograph of a preparation of the human wart virus purified by density gradient centrifugation. Normal viral particles as well as abnormal elongated forms are present. Bar indicates 0.1 micron. Potassium phosphotungstate stain.

Fig. 2. An electron micrograph of the Shope rabbit papilloma virus purified by density gradient centrifugation. Normal particles and an elongated form are present. Bar indicates 0.1 micron. Potassium phosphotungstate stain.

Fig. 3. A fluorescence photomicrograph of a frozen section of a human wart stained with fluorescein-labeled antibody prepared against the human wart virus. The human wart viral antigens are localized in the nuclei of cells in the stratum granulosum and in keratin in the stratum corneum. The stratum corneum is oriented at the top of the figure. × 400.
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