Epidermodysplasia Verruciformis as a Model in Studies on the Role of Papovaviruses in Oncogenesis

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SUMMARY

Epidermodysplasia verruciformis is a skin disease caused by a generalized infection by verruca virus in which the verrucous lesions usually change into tumors, most frequently Bowen’s carcinoma. Lesions from a case in which papovavirus was evident were transmitted to a healthy person, the virus being responsible for the verrucous lesions. This fulfilled the condition for recognition of the virus, demonstrated by electron microscopy, as being causatively involved in the verrucous lesions in epidermodysplasia verruciformis and also in the initiation of the morbid process. The virus could not be demonstrated in lesions showing distinct signs of cancer.

INTRODUCTION

EV1 is extremely valuable as a model for study of the role of viruses in oncogenesis. The skin lesions resemble generalized flat warts; they occur chiefly on the face, dorsa of the hands, and legs, and they usually persist throughout life without a tendency to regress.

The disease often runs in families and is therefore regarded as a genodermatosis. In a large proportion of cases, it leads, after many years, to multifocal neoplastic proliferation, usually of the Bowen’s carcinoma type (for detailed clinical and histological data, see Ref. 17).

As far back as 1926, some researchers (10, 19) felt that EV may be due to an infection by the verruca virus, since the lesions morphologically resemble warts. Lutz (21) reported successful autoinoculation, i.e., he elicited verrucous lesions in a patient at the site where material taken from a wart in another site was rubbed. In 1957 (17), we repeated autoinoculation and, moreover, were able to produce lesions on our own skin by inoculating crushed material from EV warts. Within several weeks, flat warts developed at the site and were confirmed histologically. In 1959 (15) we had another positive transmission from a different case of EV. The physician on whom this was done developed numerous small warts at the site of inoculation within a few months. The warts persisted for approximately 8 months, and later there was even a generalized eruption of flat warts which cleared spontaneously after a few months.

Particularly interesting studies were made of a family with EV in whom the viral character of the lesions was evident from observations of their gradual development in 2 children whose mother had EV (14). We saw here the initial verrucous lesions, even with Körner’s isomorphic irritation effect on scarification, which could not be distinguished from flat warts.

The viral character of EV has been confirmed by electron microscopy in ultrassections, as well as by the method of negative staining (1, 2, 8, 9, 11, 13, 31, 33, 34). Autoradiographic studies have also confirmed DNA synthesis in fully vacuolized cells of the stratum granulosum bordering on the stratum corneum (4, 20), similar to Shope’s papilloma and human papilloma (26, 27).

In lesions showing clear signs of cancer, the virus could not be demonstrated (1, 4, 8, 9, 11, 12, 16), although Okamoto et al. (25) reported type C, virus-like particles in rhabdosarcoma developing in EV, and papovavirus in an early carcinoma in the same case. There are also isolated reports, mainly in intraepidermal carcinoma, of virus in the upper layers of the epidermis in lesions with signs of atypia (30, 32). However, there is nothing yet to prove conclusively that the virus is the fundamental oncogenic factor, and Schellander and Fritsch (34) believe that the virus merely plays the part of the triggering factor.

The purpose of this paper is to show that the papovavirus seen in the verrucous lesions in EV is of pathogenic significance, i.e., responsible for the cutaneous lesions and not only accompanying them, and that its presence in the cell nucleus is important for the initiation of the process of oncogenesis.

MATERIALS AND METHODS

Studies were made of a 30-year-old woman (J.G.) in whom the first lesions developed at the age of 5 when her brother removed a common wart from his own skin, crushed it, and rubbed it into her skin and her older sister’s. A few months later, gradually intensifying EV symptoms developed in both girls. Two other children in this family who have always lived apart have remained healthy, and there has been no recurrence of the wart in the brother. The family has now been under our observation for over 15 years, during which time the older sister has borne 3 children, 2 of whom gradually developed EV. The patient (J. G.) has 2 healthy children. Both sisters have healthy husbands.

No malignant lesions have yet been discovered in the older sister, whereas J. G. has continued since the age of 25 to develop premalignant lesions on the forehead and then Bowen’s carcinomas, which are treated partly with...
5-fluorouracil, partly with grenz rays, and partly by surgery. The premalignant lesions have also occurred on the forearms. Skin biopsies of the forehead (Fig. 1), hands, and forearms (Fig. 2) were performed with subsequent hematoxylin-eosin staining.

**Electron Microscopy**

**Ultrasctions.** The specimens were cut into small pieces and fixed (a) in a 1% solution of osmium tetroxide containing sucrose and buffered with Veronal acetate or 0.1 M phosphate buffer, pH 7.4, at 0–4°C for 2 hr, and (b) in 3.6% glutaraldehyde with 0.1 M phosphate buffer, pH 7.4, for 3 hr, and then washed in the buffer and fixed in 1% osmium tetroxide with phosphate buffer for 2 hr.

The fixed specimens were dehydrated with ethanol in increasing concentrations and embedded in Araldit or Epon. Tissue blocks were cut in a Porter-Blum MT2 or Reichert OM-U2 ultramicrotome and examined in a Jem 7 electron microscope. In addition, the sections were stained with uranyl acetate and lead hydroxide.

**Negative Staining Technique.** The biopsy specimens were ground with sand and 0.5 ml of distilled water in a mortar and centrifuged for 10 min at 750 X g. The supernatant was centrifuged for 1 hr at 30,000 X g, and the sediment was suspended in 0.5 ml of distilled water. A drop was put on a watch glass, mixed with a drop of 3% aqueous phosphotungstic acid, and adjusted with 0.1 N sodium hydroxide, pH 6.

A small quantity of the mixture was applied to a Formvar-coated specimen grid, the excess liquid removed with filter paper, and the dried preparation examined in a Jem 7 electron microscope.

**Heteroinoculation**

Experiments were performed on 8 physicians. Homogenized material from EV verrucous lesions on the dorsa of hands was rubbed vigorously into the slightly scarified skin of the flexor aspect of both arms, and a cover glass was placed on the site for 24 hr to create a moist chamber, as described by Lutz (21).

**RESULTS**

**Histology**

In preparations from the dorsum of hands, the histology was typical of the flat warts in EV, showing distinct, basket-like, loose hyperkeratosis and pronounced vacuolar degeneration in the granular and upper squamous layer of the epidermis (Fig. 3). The corium was virtually free of inflammatory infiltrations.

Specimens from the forehead showed signs of proliferation with no indication of atypia in some lesions; there were changes of the senile keratosis type, Bowen’s disease (Fig. 4), and invasive Bowen’s carcinoma in others.

A specimen from the forearm showed proliferation with slight cell disarrangement and occasional atypical cells (Fig. 5); these changes can be described as the very onset of carcinomatous proliferation.

**Electron Microscopy**

The virus was demonstrable as crystalloid structures within the nuclei in the verrucous lesions (Fig. 6). It corresponded to viruses hitherto found in warts and EV.

In lesions of the type of Bowen’s disease on the forehead, there were enlarged nuclei with abnormally distributed chromation and, sometimes, corrugated nuclear membrane. Some cells showed 2 nuclei, and these differed occasionally in morphology. The cytoplasm was more scant in the atypical cells, whereas tonofilaments were abundant and present in irregular concentrations.

In lesions from the forearm (with signs of malignant proliferation), the cell nuclei in the upper layers of the epidermis at the border of the stratum corneum contained the virus in crystalline arrangement, with individual virus particles scattered about and occasionally indistinguishable from chromatin (Fig. 7). Chromatin was clearly less abundant in the virus-containing cells and showed a chiefly peripheral distribution. The elements of the fine structure also were less abundant in the cytoplasm of these cells.

**DISCUSSION**

EV is an acknowledged premalignant condition (3, 7, 8, 22–24, 28, 32, 33). The clinically and histologically wart-like lesions change, after a time, into tumors, primarily Bowen’s carcinoma (11, 12, 14, 32). The malignant transformation occurs exclusively in sites exposed to sunlight (32, 33), an occurrence which may suggest possible summation of carcinogenic stimuli. Electron microscopy has confirmed unquestionably the presence of papovaviruses in EV verrucous lesions. The intranuclear virus corresponds to the verruca virus in size, crystalloid character, and localization in the nuclei. The shape and size of its capsid, as established by negative staining, assign it to the group of papovaviruses, which includes also virus of warts.

In the present work, we saw viruses in proliferating lesions that already showed the characteristics of early malignant hyperplasia. This confirms reports (30, 32) of virus in lesions in which the deeper layers of the epidermis showed some
atypia without signs of invasion. In positively malignant lesions the virus could not be detected, a characteristic which agrees with the findings of Ruiter and van Mullem (32) and others (1, 8, 12, 16).

Even in the case described by Okamoto et al. (25), in which negative staining showed the virus in an early carcinoma lesion, the process was presumably not yet invasive, although their report is printed without a photomicrograph and fails to indicate the degree of malignancy. Their previous work (35) stressed the absence of virus in later stages of malignant lesions.

Transmission of the viral infection seems to supply conclusive evidence that the virus is responsible for the verrucous lesions and, consequently, is pathogenic. The verruca virus cannot be cultured in any known media or transmitted to laboratory animals. Consequently, man to man transmission can be the only proof of pathogenicity. The material for the heteroinoculation was taken from the immediate neighborhood of the lesion in which the virus, as well as some atypia, was demonstrated. This suggests that the virus also has a part in the initiation of the malignant transformation of cells. This indication is particularly distinct where virus particles are scattered without an evident crystallloid arrangement, possibly owing to the disturbed formation of the complete virus. The DNA virus seems to be incorporated in the cellular genome and, therefore, eludes detection by any method, a characteristic which is very much like the process of malignant transformation in Shope's papilloma (16, 18, 26, 27). Here the cell is transformed under the influence of the virus in long-standing infection (29), especially in the presence of factors that may cause the infection to run a particularly chronic course (5).

It can hardly be doubted that EV involves some inborn predisposition that is responsible for the eminent chronicity of the normally rather mild and easily cured condition such as infection by the verruca virus.

Currently, our research concerns immunology, especially delayed hypersensitivity in EV, since cellular defenses are basic-

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Fig. 1. Generalized skin lesions resembling flat warts on the face, neck, and trunk. On the forehead are numerous premalignant changes of the keratosis senilis type and a proliferating Bowen’s carcinoma type.

Fig. 2. Verrucous lesions on the forearm. White atrophic scar after treatment with grenz rays of an early malignant lesion.

Fig. 3. Specimen from the dorsum of the hand. Histological picture typical of the flat wart. H & E, X 180.

Fig. 4. Specimen from the flat lesion on the forehead. Histological picture typical of Bowen’s disease in situ. H & E, X 280.

Fig. 5. Specimen from the forearm. Proliferation of epidermis. H & E, X 700. Some disturbance of individual growth of the epidermal cells with clumping of the nuclei. Early stage of malignant proliferation.

Fig. 6. The cell at the border of the stratum granulosum. Virus in crystalline arrangement with some virus particles scattered about. Chromatin clearly less abundant, showing a peripheral distribution. Nuclear membrane preserved. Fine structures of cytoplasm less abundant. V, virus particles; ch, chromatin; nm, nuclear membrane; mb, myelin body. X 60,000.

Fig. 7. Crystalloid structures in higher magnification. Individual virus particles scattered about within the nucleus; some are indistinguishable from chromatin. X 145,000.

Fig. 8. Negative staining. Virus capsid with clearly visible capsomeres on the surface. X 630,000.

Fig. 9. Heteroinoculation. Verrucous skin lesions at the site about 4 months after the inoculation.
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