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Herpes Simplex Virus and Human Cancer. I. Relationship Between Human Cervical Tumours and Herpes Simplex Type 2*

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Abstract—*The presence of HSV-2 DNA was investigated in ten dysplasias and five genital tumours by the blotting technique. The sensitivity of the technique employed could reveal* 0.5 *viral genome equivalents per diploid cell genome. Viral DNA was not detected in any of the tested tumours.*

INTRODUCTION

STUDIES on the relationship between cervicocarcinomas and genital infections by Herpes Simplex Virus Type 2 (HSV-2), and other sporadic reports on labial carcinomas developing at the site of herpetic recurrences, support the hypothesis of an association between Herpes Simplex Virus and human tumours [1].

The significant association between HSV and cervicocarcinomas is supported in the literature by the fact that sera from women affected by such tumours show higher antibody titers to HSV-2 than control sera [2–4], although such a relationship has not been found in all the populations examined [3, 4]. Other substantial elements exist to support the association between HSV-2 and cervicocarcinomas. It has been shown that tumours contain not only structural viral antigens [5], but also early viral antigens [6] and non-virionic antigens [7]. A search for viral nucleic acids in human cancer cells has so far been successful only in a large (70 g) exophytic cervical squamous cell carcinoma [8]. Analysis of the nucleic acids extracted from the tumour by renaturation kinetics with labeled viral DNA revealed that only a fragment of the viral genome was present in the tumour cells and that a small portion of the fragment was transcribed. Unfortunately, such a technique requires a large quantity of pathologic material and results are severely affected by experimental conditions [9]. In fact Hansen *et al.* [10] tested human tumours for the presence of HSV DNA by the same technique, but with different experimental conditions, and obtained negative results. McDougall *et al.* [11] have examined sections of cervical cancer biopsies for evidence of HSV-specific RNA and have found hybridization of ^{3}H-labeled HSV-2 DNA to RNA in cells within areas of abnormal tissue.

In this study we analysed 50 genital dysplasias for the presence of HSV, and ten dysplasias, four cervical tumours and one vulva tumour for both the presence of HSV as infectious particles and for the presence of HSV DNA. To detect the presence of viral DNA in tumour cells we employed the blot transfer technique originally described by Southern [12]. Such a technique allowed us to work with small quantities of pathologic material. The vulva tumour was also analysed by DNA–DNA reassociation kinetics.

MATERIALS AND METHODS

Clinical tumours

For our survey, 28 cases were selected whose histological reports showed a cervical cancer, a cervical intraepithelial neoplasia or a cervical dysplasia, as indicated in Fig 1. In this figure we have also specified the relationship with the cytological report which, in eight cases, showed only inflammatory modifications. In all these

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Histology \ Cytology	PR	MD	MdD	SD	CIS	IC
M	4		2	1		
MD	3	1		1		
MdD	1	4	3	1		
SD			1	2		
CIS					1	
IC						3

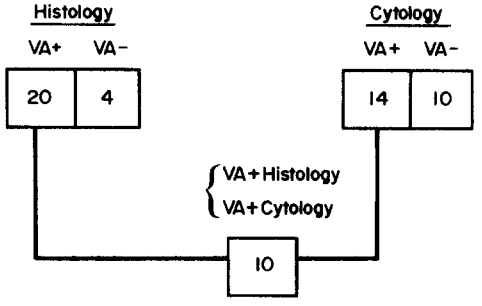

Fig. 1. Histology and cytology of selected cases, and correlation between viral alterations. VA, viral alteration; +, present; −, absent, PR, phlogistic reaction; M, metaplasia; MD, mild dysplasia; MdD, moderate dysplasia; SD, severe dysplasia; CIS, carcinoma in situ; *IC, invasive cancer.*

cases cellular alterations commonly attributed to viral aetiology were present: koilocytotic atypia, polynucleation, etc. [13–15]. Cases of condyloma were deliberately excluded, while no histological differentiation was made on the basis of the prevalence of any characteristic attributed by various authors to a supposed specific viral agent.

Research and characterization of HSV-2

Pathologic material was finely cut in Petri dishes and inoculated in HEp-2 and BHK cell cultures. Cells were observed microscopically daily to detect the presence of eventual HSV. The only HSV-2 isolated was characterized on the basis of (a) cytopathic effect (cpe) both in live and stained cultures, (b) neutralization by rabbit antisera to HSV, (c) electrophoretic analysis by SDS polyacrylamide gel of proteins extracted from virions purified in Dextran T 10 gradients.

Cellular DNA purification

Tissues were minced with scissors, homogenized in TD (25 mM Tris–HCl pH 7.4, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 5 mM D-glucose) [16] in a Dounce homogenizer equipped with a loose-fitting piston and digested from 5 to 10 hr with Proteinase K (Merck) at 50 μg/ml in 20 mM EDTA and 0.5% sodium dodecyl sulfate (SDS) at 37°C. The viscous lysate was then gently extracted three times with an equal volume of a phenol–chloroform mixture (50 g phenol + 40 ml chloroform). Samples were dialyzed first against 10 mM Tris–HCl, pH 7.5, 5 mM EDTA, 1 M NaCl, and then against the same buffer without NaCl. RNA was degraded by treatment with 50 μg/ml of pancreatic RNAse (Worthington) at 37°C for 1 hr, followed by Proteinase K digestion, two more phenol–chloroform extractions and dialysis as above.

^{32}P-labeled HSV-2 DNA probe

HSV-2 (G strain) viral nucleocapsids were purified from cytoplasmic extracts of infected HEp-2 cells by velocity sedimentation in sucrose gradients [17] and disrupted with 0.5% SDS, 20 mM EDTA and digested with Proteinase K (50 μg/ml). After two extractions with phenol–chloroform, viral DNA was furthermore purified by two cycles of equilibrium centrifugation in CsCl gradients. *In vitro* labeling by nick translation was performed as follows. ^{32}P-Labeling was carried out in a reaction mixture containing 20 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 20 μg bovine serum albumin (Sigma, fraction V), 80 μCi each of the four ^{32}P-deoxyribonucleoside triphosphates (The Radiochemical Centre, Amersham; sp. act. 350 Ci/mM), 4 μg of HSV-2 DNA and 20 units of *E. coli* DNA polymerase I (Grade I, Boehringer, Mannheim). Incubation at 18°C for 45 min was terminated by the addition of EDTA. Incorporated radioactivity (35–40% of input) was separated from residual triphosphates by Sephadex gel filtration

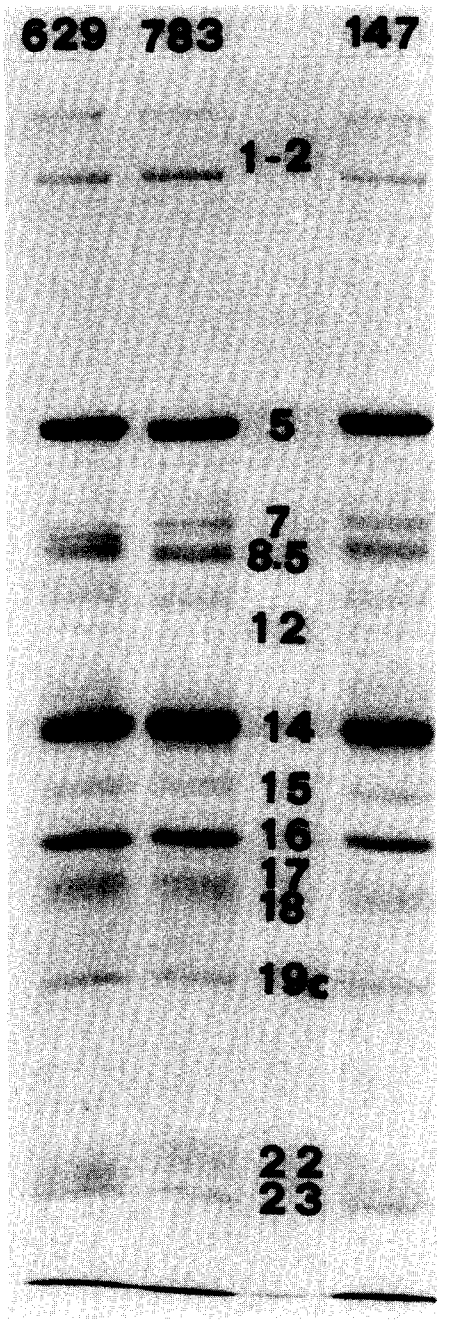

Fig. 2. Autoradiogram showing electrophoretic pattern of the HSV-2 strain (783) isolated from cancer compared to patterns of other HSV-2 strains (629, 147). The acrylamide gel electrophoresis was performed as previously described [26]. *The numbers assigned to individual HSV polypeptide bands follow the designation assigned by Cassai* et al. [27].

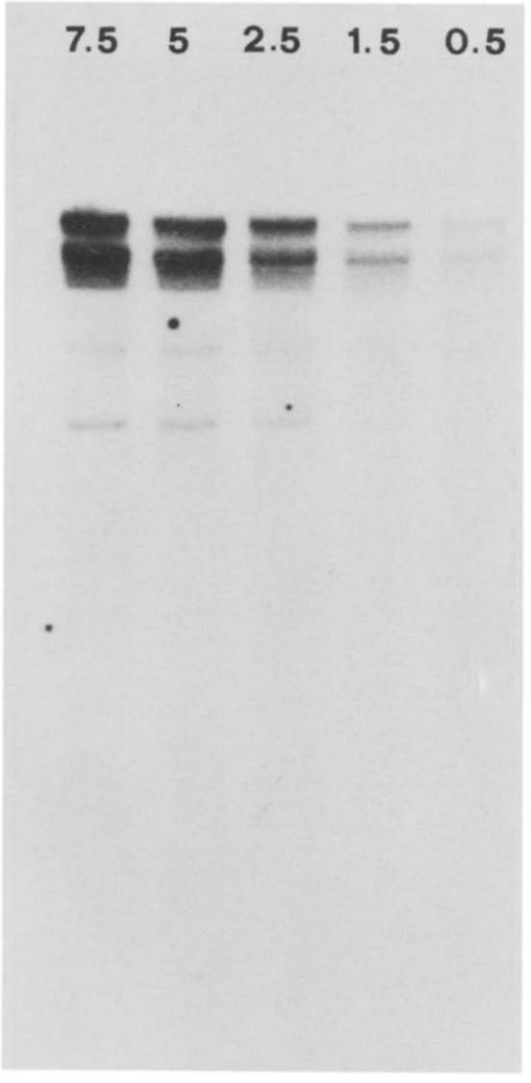

Fig. 3. *Hybridization reconstruction experiment. Purified* ^{32}P-*labeled HSV-2 (G) DNA was hybridized to nitrocellulose blots containing EcoR1 restriction enzyme fragments of HEp-2 DNA and HSV-2 (G) DNA in the ratios of* 7.5, 5, 2.5, 1.5, 0.5 *viral genome equivalents per diploid cell genome.*

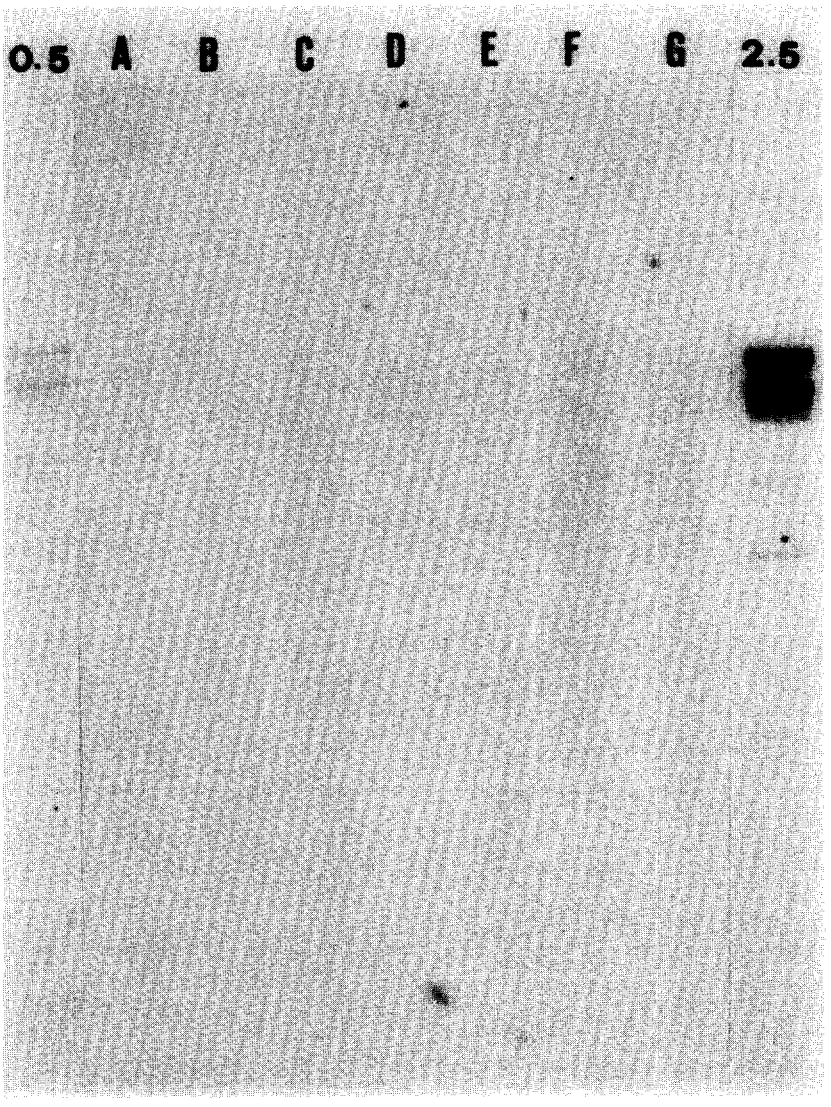

Fig. 4. *The first and the last columns show blot hybridization controls with* 0.5 *and* 2.5 *HSV-*2 *genome/HEp-*2 *cell genome; other columns* (*A–G*) *show DNAs extracted from tumour cells and hybridized with* ^{32}P *HSV-*2 *DNA.*