Hypothesis

HUMAN GENITAL CANCER: SYNERGISM BETWEEN TWO VIRUS INFECTIONS OR SYNERGISM BETWEEN A VIRUS INFECTION AND INITIATING EVENTS?

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Summary It is proposed that human genital cancer results from a "promoting" papillomavirus infection and initiating events, frequently caused by herpes simplex virus (HSV) infections. This hypothesis is based on the demonstration of DNA from different types of papillomaviruses in some cervical cancer biopsy samples and in premalignant lesions; on studies revealing the initiator-like functions of HSV infections; and on analogous interactions of papillomavirus infections with initiators in the induction of certain animal and human carcinomas. The model reconciles seroepidemiological data linking HSV to human genital cancer with the apparent difficulties in finding HSV DNA by biopsy in genital cancer.

INTRODUCTION

CONSIDERABLE epidemiological evidence has accumulated pointing to an infectious component in the aetiology of human genital cancer. Sexual promiscuity is a well-established risk factor, and there is increasing interest in the existence of marital clusters[1] and in the higher risk of genital cancer in sexual partners of women with cervical cancer and men with penile carcinoma.[2] Consequently, a search for causative agents began some time ago and today viruses appear the most likely candidates.

HERPES SIMPLEX VIRUSES IN GENITAL CANCER

There are three lines of evidence which support a possible role for herpes simplex virus type 2 (HSV-2) in the induction of cervical cancer:

(i) Women with cervical cancer have higher HSV-2-specific antibody titres than appropriately matched controls. In almost every published study the percentage of women with HSV-2 antibodies is higher in cervical-cancer than in control groups.[3] However, there has been a varying percentage of cervical-cancer patients with no detectable antibodies to HSV-2, even when measured with highly sensitive assays,[4] in most studies.

(ii) Experimental studies have shown the oncogenic potential of partially inactivated herpes simplex virus (HSV) in rodent cells.[5] Hamster and mouse cells are particularly susceptible to malignant transformation by partially inactivated HSV. There have been no reproducible reports on transformation of primate cells by HSV.

(iii) Some workers have found HSV-specific RNA in premalignant and malignant cervical tissue, as detected by DNA-RNA in-situ hybridisation,[6-8] and HSV-specific non-structural and structural antigens in tumour tissue and in premalignant lesions.[9]

The postulated role of HSV-2 in human genital cancer has been undermined by the failure to detect HSV-specific DNA in cervical-cancer biopsy samples[2] except in one case reported by Frenkel et al.[10] Even highly sensitive hybridisation assays have given negative results[11] (and L. Gissmann and H. zur Hausen, unpublished). Recently J. K. McDougall and colleagues (unpublished) found a small HSV-2 fragment, corresponding to the Bgl II N region, in biopsy samples of two of eight cervical-cancer patients and of one patient with a cervical adenocarcinoma. However, this fragment could not encode all the antigens so far found in biospy samples, and it does not cover all regions of the genome found to react in in-situ hybridisations.

Recent observations suggest a very unusual mode of interaction of HSV with host cells: HSV seem to represent potent mutagens. They are known to be efficient inducers of chromosomal aberrations,[12] probably depending on an early viral gene function.[13] The virus effectively induces cellular DNA repair,[14,15] possibly by means of a virus-specific deoxyribonuclease[16] or a DNA polymerase with additional exonuclease function.[17] The mutagenic effect of partially inactivated HSV on host-cell genomes has been demonstrated for the hypoxanthine-guanine-phosphoribosyl-transferase locus of human rhabdomyosarcoma cells[18] and is underlined by the selective gene amplification of SV40 DNA in Chinese-hamster cells after infection with active as well as with partially inactivated HSV (Schlehofer et al. unpublished). This amplification is a consistent feature of chemical and physical carcinogens.[19] These data stress the mutagenic activity of HSV and point to a role of this agent as an "initiator".[20] Such a role would reconcile the sero-epidemiological data with the lack of viral DNA in tumour biopsy samples and could also account for the efficient transformation of rodent cells which are also transformed by chemical and physical initiators.

PAPILLOMAVIRUSES IN GENITAL CANCER

The absence of detectable quantities of HSV DNA in cervical-cancer biopsy samples prompted the search for other potential agents and led to the hypothesis that papillomaviruses detected in human genital warts may have a role in human genital cancer.[21]

Human genital warts are sexually transmitted and are frequently characterised by extensive exophytic growth. Papillomavirus infections of the cervix are quite frequent and result in flat dysplastic lesions designated "atypical condyloma".[22] Papillomavirus particles have been demonstrated within koilocytotic cells of such lesions by electron microscopy.[22] The possibility that a higher proportion of cervical dysplasias reveals cytological signs of papillomavirus infection was substantiated by Shah et al.[23] who demonstrated papillomavirus-group-specific antigens in about 50% of such lesions. This clearly indicates the previously underestimated frequency of papillomavirus infections of the genital tract.

The characterisation of papillomaviruses in genital warts was rather complicated. Although the plurality of human papillomaviruses soon became apparent[24,25] the low particle concentration in genital warts permitted typing of the virus in only one exceptional case or after molecular cloning.[2,26-28] Two related types of human papillomavirus have so far been identified—type 6 and type 11. The latter virus seems also to be the prevalent virus type in human laryngeal papillomas,[27,28] thus suggesting that the mucosal epithelium is the favoured site of infection.

Although initial attempts to demonstrate papillomavirus DNA in malignant genital tumours were unsuccessful, evidence is gradually accumulating. All six invasively

growing giant condylomata acuminata (Buschke-Löwenstein tumours or non-metastasising verrucous carcinomas) so far investigated contained DNA of human papillomavirus type 6 or of a related papillomavirus[26] (and L. Gissmann et al., unpublished). Five of twenty-seven cervical-cancer biopsy samples (two carcinomas in situ and three invasive carcinomas) contained DNA of human papillomavirus type 11 or of a cross-reacting virus[28] (and L. Gissmann et al., unpublished). Green et al.[29] reported the presence of papillomavirus DNA, related to viral DNA isolated from a patient with epidermodysplasia verruciformis and distinct from human papillomavirus types 6 and 11, in two of ten vulval and two of thirty-one cervical carcinomas. DNA sequences hybridising to papillomavirus DNA have been found in two verrucous carcinomas, in one carcinoma in situ and in one invasive cervical cancer (A. J. Faras, unpublished).

Thus, genital carcinomas may contain the DNA of distinct papillomavirus types: this situation is similar to that in epidermodysplasia verruciformis, in which infection with at least four distinct, but specific, types of papillomaviruses may lead to the development of squamous-cell carcinomas at light-exposed sites.[30] Other papillomavirus types, also found in this disorder, do not seem to be involved in malignant conversion.

On the basis of these observations, one may postulate that genomes of as yet unidentified papillomavirus types may be found within those tumour cells which have been negative when tested with currently available probes. Although the presence of human papillomavirus DNA in a certain percentage of carcinomas does not prove aetiological involvement, it does provide a more solid background for the development of models to understand the role of such agents in human genital cancer.

PAPILLOMAVIRUSES AND CARCINOGENS

Under some natural, but also experimental, conditions, specific types of papillomaviruses induce papillomas with a tendency to convert into squamous-cell carcinomas: in most cases this conversion seems to require the presence of carcinogens. In epidermodysplasia verruciformis, if induced by specific types of papillomaviruses,[30] malignant conversion occurs frequently, but almost exclusively at sun-exposed sites. Many juvenile multifocal laryngeal papillomas converted into squamous-cell carcinomas after therapeutic X-irradiation.[31] It is interesting that the most prevalent papillomavirus type in laryngeal papillomas, type 11,[27] is also the prevailing type in atypical condylomata of the cervix.[28] Cancer of the alimentary tract of cattle appears to require interaction between a specific type of papillomavirus (bovine papillomavirus type 4) and the consumption of bracken fern which contains a potent carcinogen.[32] Rous et al.[33] reported the synergistic effects of chemical carcinogens in the rapid and efficient conversion of Shope-papillomavirus-induced papillomas to carcinomas in domestic rabbits.

Thus, the ultraviolet part of the sunlight in epidermodysplasia verruciformis, X-rays in laryngeal papillomatosis, and chemical carcinogens in oesophageal papillomatosis of cattle and in the malignant conversion of Shope papillomas in domestic rabbits, share mutagenic and initiating properties. They appear to be important or even essential components in the development of malignant tumours. Neither target (whether viral or host-cell DNA) nor mechanism of this interaction is yet known. The reproducibility of these observations, however, is the basis for my hypothesis on the pathogenesis of human genital cancer.

MODEL FOR SYNERGISTIC EFFECTS IN HUMAN GENITAL CANCER

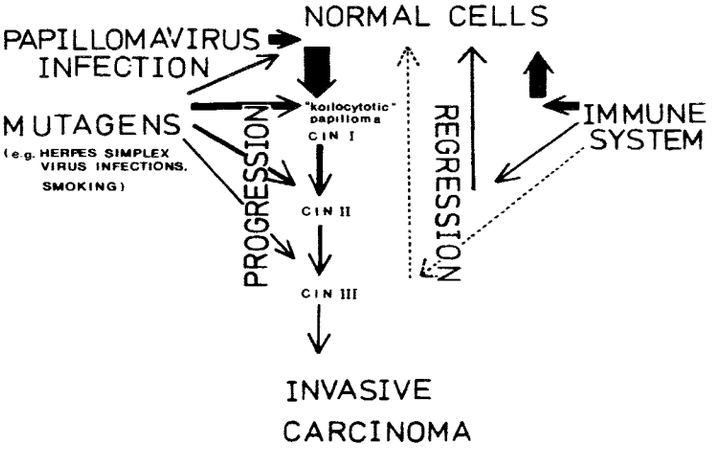
The assumption that papillomaviruses play a role in the induction of genital cancer is presently based on the frequent demonstration of papillomavirus particles, antigens, and nucleic acids in cervical dysplasia, and the demonstration of papillomavirus DNA in a limited number of malignant tumours. It is assumed that as yet unidentified papillomavirus genomes will be discovered within "negative" genital tumour cells.

Is it possible to define initiating events which could interact synergistically with these types of infection? The recent demonstration of the mutagenic potential of HSV infections[18] (and Schlehofer et al., unpublished) seems to fit very well into this hypothesis and would reconcile seroepidemiological data with the lack of HSV DNA sequences in tumour biopsy samples. Initiating events would occur at high frequency in recurrent herpetic infections and would substantially increase the risk of malignant conversion of HSV-infected papillomas. As a consequence, women with cervical cancer should have a higher prevalence of antibodies to genital herpes viruses. In addition, as a result of repeated antigenic exposures, they should have higher antibody titres than appropriately matched controls.

This model implies moreover that HSV infections do not represent the only condition for genital-cancer development and that other initiators would exert a similar effect in tissue predisposed by papillomavirus infection. Indeed, several studies show that heavy smoking significantly increases the risk of cervical cancer.[34] Other initiating events affecting the genital tract may still await identification.

The essential features of a model reconciling most sero-epidemiological and experimental findings in human genital cancer are outlined in the accompanying figure. Infection of normal cells with specific types of papillomaviruses leads to proliferating papilloma cells. Their progression to higher degrees of cervical intraepithelial neoplasia and finally to an invasive cancer clone is mediated by initiating events, such as HSV infections and smoking. Regression of the lesions would become less likely as the process progressed and the immune system became less effective.

This model requires further experimental substantiation. If correct, it may provide a useful working hypothesis for the pathogenesis of other squamous-cell carcinomas, particularly bronchial, laryngeal, and oesophageal carcinomas, since



Model of the synergism of papillomavirus infection and initiating events in genital cancer resulting in an invasively growing cell clone.

CIN I–III indicate stages of cervical intraepithelial neoplasia.

koilocytotic epithelial changes very similar to condylomatous lesions of the uterine cervix have been reported in adjacent tissue.[35]

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Reviews of Books

Clinical Epidemiology

The Essentials. Robert H. Fletcher, Suzanne W. Fletcher, Edward H. Wagner. Baltimore and London: Williams & Wilkins. 1982. Pp. 223. £10.50.

IT is a quarter of a century since John Paul brought the concept of population to the ward—in his book *Clinical Epidemiology* (University of Chicago Press, 1958). Although the apposition of "clinical" to "epidemiology" has been to some bulls a very red rag, a steady stream of books on this area of epidemiology is now flowing into the bookshops. The nature of much recent epidemiological research, which perhaps indicates that clinical epidemiology has generally gained acceptance as a subject, allows this book to be much more clinically orientated than was Paul's template. For instance, when the authors begin the chapter on Treatment with "Once the nature of a patient's illness has been established and its expected course predicted, the next question is: what can be done about it?", they are not contributing to the survival of a well-roasted chestnut. They are introducing their section on how to go about deciding scientifically rather than intuitively whether a well-intentioned treatment does more good than harm. Their other chapter headings are also splendidly concise (for example, Abnormality, Cause, Change, Risk), and the way in which these matters are addressed is equally succinct, clear, and free from jargon. Indeed, it is remarkable how three authors have achieved such homogeneity of style. Nearly all the research material adduced for purposes of illustration is, to my knowledge, new to an epidemiological textbook, and all of it is directly relevant to patient management—for instance, the effect of cytarabine on herpes zoster; toxic shock syndrome and tampons; the Hypertension Cooperative Group Study; and the controversial study of hypoglycaemic agents undertaken by the University Group Diabetes Program, the validity of which is analysed in detail. Although the framework in which these research findings are described is new, epidemiological principles and methods are treated systematically and soundly. Sampling, bias, when randomised trials are and when they are not appropriate are all discussed, as are such practical issues as obsolescence of a cohort, and the difficulties of studying a rare disease (but the authors must know that retrospective studies and case-control studies are *not* the same and they should also know that rare diseases have, by clever use of records, been effectively studied prospectively).

£10.50 may seem a lot to pay for a 200-page paperback, but this is a very good book, which is attractively printed and bound. It is perhaps too detailed for some undergraduate medical students, but it will be valuable to clinicians who propose to use epidemiological techniques in their research, to teachers who want to keep themselves up-to-date, and to trainees in community medicine.

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Lymphomas Other Than Hodgkin's Disease

Edited by A. E. Stuart, University of Newcastle upon Tyne, A. G. Stansfeld, St Bartholomew's Hospital, London, and I. Lauder, University of Leeds, for the British Lymphoma Group. Oxford: Oxford University Press. 1981. Pp. 69. £12.50.

SELF-HELP groups tend to consist of people who find themselves wrestling with a common difficulty and whose needs are covered inadequately by the estabalishment. So it is with the British Lymphoma Pathology Group, which was founded in 1974. The founders, and subsequent members, are all pathologists who are committed to a special interest in lymph-node pathology. This book has arisen out of the requirement for criteria on which to base the recognition of the different cell types seen in non-Hodgkin's lymphomas and for agreement as to the number of diagnostic categories needed for their subdivision. It is not wedded to any one particular classification, and the various classifications in current use are given without further comment in an appendix, although the U.S. National Cancer Institute's working formulation of non-