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# EDITORIALS

## Cancer Causes Revisited: Human Papillomavirus and Cervical Neoplasia

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In a 1989 editorial in the *Journal*, Henderson (1) commented on the state of the evidence concerning viruses and human cancers and concluded that, of the possible causal associations examined by epidemiologic studies, that of the hepatitis B virus with liver cancer was the one bolstered by the most indisputable body of evidence up to that point. He pointed out that important inconsistencies remained in our knowledge of the association between the human papillomaviruses (HPV) and cervical carcinoma and that these inconsistencies must be resolved before we could accept HPV as having a causal relationship with this cancer. His remarks had been prompted by an article published in the same issue of the *Journal* (2) that raised questions about the sexual transmissibility of cervical HPV infection.

Since the late 1960s, epidemiologists had dutifully documented that cervical cancer was consistently associated with sexual behavior (3), and they expected that the purported sexually transmitted agent behaved as such, i.e., that sexual practices explained its distribution in the population of women at risk. At that time, the results of two other large-scale studies (4,5) further fueled the controversy. One (5) failed to demonstrate the sexual transmission of HPV infection in the general population, and the other (4) found only a moderate association between detection of the virus and risk of cervical cancer in a multi-center case-control setting, with relative risks in the 3-4 range. In the case of hepatitis B virus and hepatocellular carcinoma, the magnitude of relative risks are in the double digits, with one cohort study (6) unveiling a greater than 100-fold elevation in risk among carriers. It is very hard to argue against a causal association in the face of relative risks of such a high magnitude.

What those investigations of HPV and cervical cancer had in common was the method for detecting the virus in cervical specimens—the filter in situ hybridization technique, which had been proposed in the mid-1980s as an accurate HPV DNA detection tool amenable to application in large-scale epidemiologic investigations. This assay is now known to have inadequate specificity and sensitivity, and its use results in considerably attenuated estimates of effect for the relationship between HPV infection and its putative antecedents (sexual activity) or consequences (cervical neoplasia) in epidemiologic studies, as could be demonstrated theoretically (7) and empirically (8).

Now, 6 years after Henderson's sobering editorial (1), a new generation of truly sensitive and specific molecular biology techniques has been used by epidemiologists to study the relationship between HPV and cervical cancer. Some landmark studies have appeared, demonstrating that HPV infection is highly predictive of subsequent cervical intraepithelial lesions in cohort studies (9) and that it is strongly associated with both low-grade and high-grade lesions (10) and with invasive carcinoma (11) in case-control studies, with relative risks being in the range of 20-70. Further coherence and plausibility to this etiologic model is brought by the recent findings that HPV infection fits neatly the profile of a sexually transmitted virus (12,13). Add to that an impressive body of experimental evidence from laboratory studies [reviewed in (14)] and virtually all classic criteria used to ascertain causality in chronic diseases can now be considered satisfied.

The scenario of an easily diagnosed viral infection as the precursor event leading to cervical cancer calls for action on two fronts: primary prevention by immunization against HPV and secondary prevention by augmenting cytology screening with testing for cervical HPV infection. Bosch, Manos, and co-workers (15) acted on the basis of these premises when they set out to conduct their international study of HPV in cervical carcinomas, i.e., that proper characterization of the distribution of the various HPV types in cervical cancer from different geographic areas "is essential to the development of vaccination strategies to curb the burden of cervical cancer." Before their study was conducted, it had been known that detection of HPV DNA in cervical carcinomas was more the norm than the exception, with rates in the 60%-90% range, depending on the type of tumor specimen tested (biopsy or surgical specimen), the mode of preservation (fresh, frozen, or paraffin-embedded), and, expectedly, the detection method (hybridization or polymerase chain reaction [PCR]). Few studies were large enough or representative of entire geographic regions, and this limited the generalizability of prevalence estimates. The greatest concern, however, was with the variation in laboratory methods, with

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their different levels of sensitivity and specificity. Bosch, Manos, and coworkers (15) used a PCR protocol based on consensus primers flanking a relatively conserved region in the L1 gene of HPV. This technique, also known as the MY09/11 protocol (16), has now been used extensively in epidemiologic studies and is among the most sensitive and specific for detecting and typing HPV in biologic specimens.

Is there a subset of cervical cancers that are truly induced by carcinogenic routes other than that of HPV infection? Are HPV-negative cervical cancers perhaps reflecting loss of the HPV genome as the disease progresses? Those attempting to discover genuine HPV-negative cervical carcinomas face as daunting a task as those searching for viral DNA in organs or tissues (visceral sites) where HPV is not expected to be present. In the former case, investigators will have to demonstrate that failure to detect HPV DNA is not due to insufficient sensitivity, whereas in the latter case, they will be hard pressed to prove that an occasional HPV-tissue association does not result from insufficient specificity or contamination. Bosch, Manos, and coworkers (15) found, on initial testing, a prevalence rate of 87%. Their meticulous assessment of the lot of specimens remaining HPV negative revealed that HPV DNA could be detected in separate portions from the same specimen, which raised the prevalence to 93%, and even higher than 95% on reanalysis with PCR using other primers. A concern that has been frequently voiced is whether HPV-negative cervical tumors may contain as yet unidentified HPV types that may be missed by PCR protocols in current use. It is reassuring that after testing nearly 1000 tumors—the largest and most representative series to date—Bosch, Manos, and coworkers found only one new HPV type. It is also reassuring that the proportion of HPV-negative specimens did not seem to vary according to stage of disease, which speaks against loss of detectability due to progressive acquisition of malignant features by the tumor.

The article by Bosch, Manos, and coworkers (15) can be viewed as a critical contribution to our understanding of the etiology of cervical cancer. Given that traces of HPV were detected in more than 95% of all cervical cancers and that the few HPV-negative tumors may represent instances of decreased detectability, one must entertain the possibility that HPV infection may turn out to be the first cause of a human cancer shown

to be a necessary one. Neither tobacco smoking nor hepatitis B virus have attained such a high pinnacle as risk factors for the cancers with which they have been associated.

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