Possible Etiologies of Cancer of the Cervix Other Than Herpesvirus

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Summary

Various hypotheses concerning both infectious and noninfectious causes have been proposed for the etiology of cervical cancer. The evidence for noninfectious agents and for infectious agents other than herpesvirus is reviewed here. Although some carcinogenic potential for smegma may be inferred from experimental studies, epidemiological studies do not indicate a significant role for either smegma or circumcision in human cervical cancer. Coppelson and colleagues have developed a hypothesis to explain the importance of early coitus in cervical cancer, proposing that sperm is the nucleic acid vector and the potential mutagen for tissues undergoing active metaplasia. The most attractive aspect of this hypothesis is the suggestion that any sperm may be a potential mutagen, not because of some special intrinsic property but because of a particular susceptibility of the target tissue at certain times.

Six infectious agents have an established association with cervical cancer. The association with Trichomonas, syphilis, and gonococcal infections in all probability merely reflects the fact that these infections are strongly associated with promiscuity. The correlation of mycoplasmal infection with mild cervical dysplasia, as well as with promiscuity, calls for more serious consideration of this infection, but there is no consistent association between mycoplasmal infection and cervical cancer. Chlamydial infections may be important because of their chronicity, their association with chronic inflammatory disease, and their venereal mode of transmission. Cytomegalovirus infections may be implicated upon similar grounds. This virus is known to be associated with chronic inflammatory disease, but evidence for a venereal mode of transmission and for male genital infection is less convincing. Whatever the evidence for the association of these agents with cervical cancer, none appears to be so likely a candidate for its cause as herpesvirus type 2.

However, future analytic studies should include examination of male partners and the study of semen for infectivity and such studies should be designed to test multifactorial hypotheses. Too often our hypotheses are single-agent oriented; the design and analysis of studies should both be structured to study multiple possibilities.

Any consideration of etiological hypotheses for cervical cancer must begin with consideration of the epidemiological characteristics of that disease. These characteristics, which have been summarized by Rotkin (44), are now universally accepted, since there is remarkable uniformity among studies of diverse populations done at different times (Table 1). Thus, at highest risk of cervical cancer are women who have had 1st coitus at an early age (43), have had multiple sex partners, are sexually promiscuous (50), and are of lower socioeconomic status (28). Any hypothesis must also account for those women at lowest risk, in particular the celibate (14), and to a lesser degree the nulliparous and Jewish women (50). There are many further refinements which might be made to a list of positive and negative risk factors, such as early marriage, early pregnancy, multiple marriages, and multiple pregnancies (55), some of which appear more important in some studies than in others, but the most important variables are generally accepted to be the 1st ones stated.

Smegma and Circumcision

The remarkable difference in risk of cervical cancer between Jewish and non-Jewish women, and the undeniable decreased risk of penile carcinoma in Jewish men, resulted in a series of studies designed to test the hypothesis that circumcision was the key factor in decreased risks and furthermore that smegma was the culprit, either as a carcinogen itself or as a vehicle for an infectious agent which was carcinogenic.

Rotkin has already reviewed the series of studies that showed that not only were women surprisingly ignorant of the circumcision status of their sexual partners (27) but that there was, in fact, no relationship between cancer of the cervix and the circumcision status of the husband, when that status was objectively measured (1). The same conclusion was reached in a recent study by Terris et al (49), which also showed that there was no association between accumulation of smegma and cancer in sexual partners, even though accumulation of smegma was correlated with degree of circumcision. Comparisons in other circumcized populations, such as Moslems, have been contradictory, perhaps because of problems with assessment of disease. Despite negative epidemiological evidence of association between smegma and cervical cancer, there have been attempts to examine the biological basis for such a hypothesis. One approach tested the carcinogenic potential of smegma experimentally in an animal model; another looked for infectious agents in this material.

The experimental animal models have yielded variable results. Initial experiments by Fishman et al. (11) and later
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experiments by Reddy and Baruah (42) were direct and were essentially negative, using human smegma in mice. Plaut and Kohn-Speyer (38) used a buried skin tunnel and horse smegma (for larger quantities). They produced tumors in a small proportion (6 of 190) using this skin tunnel but not with s.c. inoculation. The most thorough examination of these experimental models was by Pratt-Thomas et al. (20, 39) in which human smegma was used in a diverse group of experimental mouse models. Significant findings resulted when smegma was introduced into the vagina biweekly for more than 14 months. For the few mice that survived, carcinoma or epithelial hyperplasia resulted. The significance for human cancer of such models in a susceptible mouse strain (DBA/1) remains unsettled, although some carcinogenic potential appears to have been established.

Although the experimental evidence that smegma is carcinogenic in mice may be valid, the hypothesis that smegma is the carcinogenic factor in human cervical cancer does not adequately account for all of the epidemiological characteristics already cited. However, if the concept that there are variable periods of susceptibility of the cervix to a carcinogen is also considered, smegma as the vehicle of a carcinogen might be a more attractive hypothesis. In an attempt to combine the hypothesis of viral causation with one of smegma as a significant factor in the decreased risk of cervical cancer in Jewish women, Rawls et al. (41) in 1968 tested smegma for evidence of viral infection. Only 4 of 220 smegma specimens were found to yield virus, in all instances herpesvirus, and only from the 4 young men with penile lesions in the study.

Thus, although some carcinogenic potential for smegma might be inferred from experimental studies, there is little epidemiological support for its significance in human cervical cancer and even less for the significance of circumcision.

Sperm

An alternative hypothesis to explain the importance of early coitus in cervical cancer has been developed by Coppleson and Reid (9, 10). A basic precept in this theory is that there are dynamic phases in the history of the evolution of the squamous epithelial coverings of the cervix known as metaplasia (Table 2). These occur most actively in the early (postpubertal) period and 1st pregnancy and are recognized to be phases of active cell growth with phagocytosis, pinocytosis, and nucleic acid turnover. The risk of occurrence of atypical metaplasia and consequent risk of dysplasia are highest in periods of most active physiological metaplasia. In experimental animal studies and in limited observations of humans shortly after coitus, sperm DNA has been visualized in intimate association with the cell nucleus. Thus sperm is proposed as the significant nucleic acid vector, and potential mutagen, for tissue undergoing active metaplasia. The most attractive aspect of this theory is the suggestion that any sperm might become a potential mutagen, not because of any specific intrinsic property of that individual source but because of the particular receptivity of the target tissue. Obviously, sperm is a more common and more abundant source of DNA for incorporation into cervical epithelium than more transient and less evident viral sources (e.g., herpes simplex virus type 2). Whether or not there are males at higher risk of evoking cervical cancer in their partner is an unsettled question. Martínez (29) described an unusual incidence of cervical carcinoma in partners of men with penile cancer.

Supporting the sperm theory, and basic to the understanding of the meaning of the epidemiological determinants of cervical cancer, is the concept that there may be varying periods of receptivity of cervical epithelium to potential mutagens. One such period is early adolescence and another is 1st pregnancy. At these times the cervical epithelium is most susceptible to some concomitant of coitus. Although it may be sperm itself there are other possibilities that should be explored. That early coitus induces atypical metaplasia has been clearly shown by the Coppleson group (9) in a study of promiscuous adolescent girls, in whom atypical transformation zones were found in much higher frequency than controls.

In addition, this hypothesis attempts to explain the differential risk in Jewish women by suggesting that Jewish religious doctrines are contributory to that low risk. Abstinence from coitus during menses and immediately following, during certain antepartum and postpartum periods, and during any vaginal hemorrhagic discharge are believed to be crucial. Such hypotheses must be tested in real life (55) and are presently the basis for a large collaborative study in Australia (47).

Infectious Agents

Although a major effort is currently underway to evaluate herpesvirus as a single etiological factor, it is worthwhile to review other agents of cervical infection for their potential in this regard, both as competitors for such solo limelight, or as members of a group of agents with a range of carcinogenic potential. Candidates for consideration should be relatively common inhabitants of the female genital tract, be implicated in venereal transmission, and be compatible with the epidemiological determinants of cervical cancer. Historically, agents have been considered as possible etiological factors simply because of their recovery in the cervix, with or without manifest disease (e.g., adenovirus, Staphylococcus, Streptococcus, bacteroides, etc.), sometimes because of their implication in disease states resembling cervical cancer [e.g., tuberculosis, schistosomiasis (5), and amebiasis (30)] and sometimes because of their recovery from cervical cancer [e.g., Döderlein's bacillus (36)]. I have selected only 6 candidates for further

Table 1

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<tr>
<th>High risk</th>
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<tr>
<td>Early coitus</td>
<td>Celibacy</td>
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<tr>
<td>Multiple sexual partners</td>
<td>Nulliparity</td>
</tr>
<tr>
<td>Promiscuity</td>
<td>Jewish religion</td>
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<td>Lower socioeconomic status</td>
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discussion as they best fulfill the criteria listed above (Table 3). There is no simple and overwhelming association of any single agent to date. So it is clear that there is a single agent yet to be recognized, that there are multiple influences of a number of agents, or that the association is an indirect and complex one.

I review the selected agents in Table 3 in reverse order. **Trichomonas.** As is true of many of these infectious agents, the historical origin of a concept of association between cervical cancer and *Trichomonas* probably arose from the similarity between certain severe cases and early cancer (23). Furthermore, *Trichomonas* is an established pathogen of male and female genital tracts and is transmitted sexually. Furthermore the epidemiological characteristics of infected women are very similar to those of cervical cancer in some of the careful studies that have been conducted (6, 34). Many indices of socioeconomic status show an inverse correlation and there is increased prevalence of infection with sexual activity, with highest frequency in the early married. Only in recent studies in Israel is there a dissimilarity between the epidemiological pattern of the 2 diseases (4). Here a higher infection rate is noted among the Western- and young Israeli-born women as compared with those originating from Eastern countries, despite the fact that a higher standard of living, higher educational background, and better sanitary conditions exist in the former group. This apparent contradiction may be possibly due to a greater promiscuity in the former group, but studies of these factors have not yet been made. Cervical cancer cases have been reported more frequently in residents and immigrants from Eastern countries (40), but it may be that this trend will reverse in subsequent years. Finally, a consistent finding in most studies of trichomoniasis is an association with cervical atypia (6, 32, 34) and in some cases with invasive carcinoma (3). In this treatable disease, regression of cervical dysplasia and hyperplasia can be seen with therapy (4). On the one hand this supports a causal hypothesis for the association of *Trichomonas* and the cytological abnormalities, but alternatively it may suggest that these abnormalities are, in fact, not truly precancerous. This is worth stressing because for a number of other agents, such as herpesvirus, where treatment is less feasible, it is often not possible to establish the eventual significance of the associated cytological abnormalities without interruption (e.g., conization); and there is considerable doubt that such observational outcome studies are ethically justifiable today.

In summary, there is an association of *Trichomonas* and cervical cancer that is still worthy of evaluation and study, although few researchers suggest that it could account for a significant proportion of cervical cancer and that it probably reflects some other covariable of promiscuity. **Syphilis.** The relation between syphilis and cervical cancer was one of the earliest of specific associations to be noted. The epidemiological characteristics of this venereal disease were similar to those of cervical cancer, although the rather marked changes in incidence that have occurred in recent decades in the United States have not been reflected in the changing incidence of cervical cancer.

Most of the studies that showed an important association of cervical cancer and past history of syphilis (usually serological) are older studies when syphilis was more frequent (26). Some of the most recent epidemiological studies in the United States have failed to show this association (55). This is not true in other countries and in our own current studies of cervical cancer in Taiwan we find...
a prevalence of 25% seropositivity in 150 cervical cancer cases as compared with 10% each in 2 groups of matched controls of equal size. There is some association of syphilis with cervical atypia in these studies; it is seen in recent studies in the United States among high-risk groups such as prostitutes or among prison populations (33, 37). Syphilis is relatively rare as a cause of chronic cervical infection, and thus there is little support for any serious etiological hypothesis for this agent. It is presumed that history of syphilis reflects sexual promiscuity and is of major significance in identification of persons at higher risk of cervical cancer.

Gonorreaa. Although this acute and recurrent venereal infection similarly may be useful in designation of high-risk persons, no specific association of infection and cervical cancer has been proposed. Perhaps this is due in part to the difficulty in recognition of infection in women, which is often asymptomatic. The fact that serological methods are presently inadequate to define asymptomatic or past infections obviously hinders the association of infection in early life with subsequent risk of cancer.

Worthy of mention at this conference is a possible interaction between herpesvirus and Neisseria gonorrhoeae Beiby et al. (2) have suggested that gonorrhea may be influential in activating latent cervical herpes in much the same manner that acute upper respiratory infections activate latent facial or oral herpes. Certainly in recent studies of female genital infections in Seattle venereal disease clinics, an unusual association of N. gonorrhoeae with both herpesvirus and with Chlamydia was seen (54), but longitudinal studies are needed to evaluate the significance of such cross-sectional findings.

Mycoplasma. The association of both T strain Mycoplasma and Mycoplasma hominis with sexual activity is well documented. Such evidence is based on a higher prevalence in venereal disease clinics than in other clinic populations (17, 21) and on studies in prisoners (12) and among promiscuous persons (48). One of the most convincing studies in this regard was recently reported by McCormack et al. (31), who showed that the prevalence of either of these strains in young girls increased significantly with history of sexual intercourse and was even higher with history of more sexual partners. In our own studies in venereal disease clinic patients in Seattle (18) the cervical prevalence of T Mycoplasma reaches 93% in women with cervicitis and for M. hominis it was 54%. Incidentally, there was a statistical association between each organism and cervicitis as there was for gonococcus and Chlamydia, but the etiological significance of Mycoplasma agents is yet uncertain. For males the association of genital pathogens and nonspecific urethritis is far more clear-cut. Only chlamydia show such a statistical association (45% recovery for the disease and 3% for controls (21)). On the other hand, our studies have not shown an association between Mycoplasma and nonspecific urethritis as others have (46), and we feel that the pathogenicity of Mycoplasma for the genital tract is much more in doubt. Of particular interest is the recent report of recovery of Mycoplasma from seminal fluid in males well correlated with recovery from the cervical secretions of their partners (15).

Besides the relationship of Mycoplasma infection to sexual activity, there are inconsistent but rather convincing reports of correlation with mild cervical dysplasia (17). In our own studies such correlation has been seen only for cervical atypia and with not more significant dysplasia. Again, as with some of the other infections reviewed, data so far have been derived from isolations and not serological studies.

Chlamydia. Organisms of this genus are subdivided into 2 species, and we are concerned with Subgroup A (Chlamydia trachomatis) as genital pathogens (8, 22). The advent of improved methods of cell culture (16) and of antigenic differentiation of strains (53) has recently altered concepts of the prevalence and variety of these infections in both men and women. Unfortunately, although there have been parallel advances in serological methodology (52), they are still too complex and cumbersome to be practical as epidemiological tools.

In our recent studies of venereal disease clinic patients in Seattle (18), chlamydia were more than twice as prevalent as herpesvirus in the cervix (but less than Mycoplasma or gonococcus) and they were associated with cervicitis (26% recovery in 100 cervicitis cases versus 11% in controls). As cited before, in males, chlamydia were rarely recovered from males in the absence of symptoms of urethritis. Preliminary information on serotyping of these strains by S.-P. Wang (personal communication) has shown a broad spectrum of types, including ocular trachoma and LGV² types as well as those typically genital. For example, of 40 strains recently isolated from cervices in Seattle, 75% were of the types now designated as genital trachoma-inclusion conjunctivitis types. But 5 (12.5%) were of serological types that are characteristic of ocular trachoma and another 5 (12.5%) were of a serotype that has been designated LGV-III (22, 24). LGV-III is an LGV strain but in these instances was not associated with the classic disease. The interest in chlamydial infections is related to their chronicity, their association with chronic inflammatory disease, and their venereal mode of transmission. Too little is known from the limited number of studies in San Francisco (45), London (22), Taipei (Ref. 8; J. L. Gale, F. C. Wang, and M. C. Hsieh. Microorganisms of the Female Genital Tract in Chinese Populations, submitted for publication to Chinese Journal of Microbiology) or Seattle to be able to characterize the infection epidemiologically, although its relation to sexual activity is well recognized. In our own studies we have not seen a clear-cut relationship of cervical infection to dysplasia. Naib (35) noted some tendency to endocervical atypia in a group of 54 women, but the observation relied on cytological definition of infection only.

Cytomegalovirus and Herpesvirus Type 2. Cytomegalovirus must be considered in this review because it is the cause of an extremely chronic cervical infection, which in at least 1 of our study areas (Taiwan) has a clear association with chronic cervicitis (22 versus 8% in controls). In our Seattle studies both isolation and serological data show that the

1 The abbreviation used is: LGV, lymphogranuloma venereum.
prevalence of this cervical infection among venereal disease clinic patients (16%) (18) is far greater than among middle socioeconomic pregnant women (2%). This is not to say that cytomegalovirus infection is so clearly a venereal disease as the previous infections considered. We have studied the epidemiological characteristics of this agent in the Seattle population and find past infections to be related to age, race, education, and marital status but have not yet completed analysis of the relation to promiscuity. One study using indirect hemagglutination as a measure of past infection (13) found no association between cancer in situ and hemagglutinating antibodies, but another recent study in Denmark showed a weak association (51).

One of the enigmas surrounding epidemiological studies of cytomegalovirus has been the infrequent recovery of this virus from the male urethra. For this reason the recent case report by Lang and Kummer (25) of repeated recovery of cytomegalovirus in higher titers from semen than from urine is important. We have confirmed this observation in 2 instances in our own laboratories and are attempting to define the frequency of isolations from this source. It is on this subject that I wish to make my only comment regarding herpesvirus type 2. Other reports in this conference have attested to the reasons why this virus is a prime candidate as an etiological factor in cervical cancer. It fulfills most of the criteria for such a candidate. Somewhat disturbing has been the low frequency of isolations from certain population groups, although serological studies confirm that the infection is more common in a lifetime of experience. However, as with cytomegalovirus, the more infrequent recovery of herpesvirus from the male urethra has continued to perplex investigators. A recent report of Centifanto et al. (7) suggests that here again we should be looking at semen or prostatic fluid. In that, they are undoubtedly correct but we cannot agree with their conclusion that their study “indicates that the male genitourinary tract is a reservoir for herpes.” Although our laboratory has not examined any large number of prostatic fluid or semen specimens for herpesvirus, we have searched for the virus in the male urethra of venereal disease clinic patients in Seattle and recovered it in less than 2% of 92 patients serially examined. Likewise, J. L. Gale and J. M. Gordon (Urethritis in a Male U. S. Military Population in Taiwan, submitted for publication to the Journal of the Formosan Medical Association) recovered herpesvirus from only 2 of 512 male servicemen in Taipei. These results are markedly different from the 8% urethral isolates reported by Centifanto et al. (7) and suggest that the sample that they studied might be unique. We have also attempted without success to recover herpesvirus from 25 vas deferens specimens taken at vasectomy, whereas they reported recovery in 9 of 31 instances. Nevertheless, their report is important in drawing attention to these specimens as a source of virus.

Conclusions

If the study of etiological hypotheses for cervical cancer is to be pursued vigorously, what points of emphasis have been developed in this review?

Perhaps immediate concern might be given in retrospective studies to a more thorough examination of male sexual partners of cancer cases. Also, prospective studies of female sexual partners of infected males should be instituted, since the concept of infective semen fits well the agent-donor concept in early coitus.

The length of the latent period is a recurrent problem that arises in all attempts to interpret the role of infection in cancer. If the epidemiological clues are correct, we are primarily interested in infections of the early years of life, and thus recovery of agents upon recognition of cancer is likely to be irrelevant, and serological evidence of past infection may be muddled by the background noise of 20 years of intermittent exposure to various agents. Perhaps the most important clues will be learned from study of infections of the young, in particular of high-risk groups, and from some streamlined prospective studies, since a prospective study of cervical cancer of a classical nature is unjustifiably cumbersome and expensive.

Finally, it would appear logical to study some truly multifactorial hypotheses of causation, particularly in regard to the significance of infectious agents. It is unlikely that 1 single factor will be discovered to explain the occurrence of cervical cancer. It is far more likely that the critical factor is the manner of host-agent interaction at a particular point in time. The agent itself may be 1 of a number of potential carcinogens. Too often our hypotheses are single agent in type; the design and analysis of studies should be structured to study multiple possibilities.

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