

# Viruses, chemicals and co-carcinogenesis

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**The etiology of cancers appears to be complex and multifactorial. Peyton Rous and others demonstrated the process of co-carcinogenesis by exposing rabbits to a virus and tars. Epidemiologists have proposed virus–chemical interactions to cause several cancers. For example, one might propose that the etiology of cervical cancer results from a complex interplay between oncogenic viruses and cervical tar exposures through tar-based vaginal douching, cigarette smoking, and/or long-term cooking over wood-burning stoves in poorly ventilated kitchens. Hepatocellular carcinoma may result from the joint effects of viruses and hepatotoxic chemical carcinogens. Kaposi’s sarcoma might happen following reciprocal actions of human herpes virus-8 infection, immunosuppression, and chemical exposures, such as nitrite radicals and alumino-silicates. Use of Koch’s postulates will not help one prove or disprove a multifactorial causation of disease; new criteria are needed. Delineating the web of causation may lead to additional strategies for prevention and treatment of several cancers.**

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## Introduction

Foremost among the pioneers of viral oncology was Peyton Rous, MD, who in 1911 described a sarcoma that could be transmitted from chicken to chicken by inoculating a cell-free filtrate, later identified as the Rous sarcoma virus (RSV) (Rous, 1911). Subsequently, Rous and colleagues demonstrated the joint action of tars, methylcholanthrene or benzanthracene, and Shope papillomavirus to consistently induce squamous cell carcinomas in rabbits (Rous and Beard, 1934; Rous and Kidd, 1938; Rogers and Rous, 1951). These historic experiments were among the first to induce cancer in animals through co-carcinogenesis. Subsequently, several scientists have recreated tumors and cancers in animal models using a variety of viruses and chemicals

in combination (Berenblum, 1954; Duran-Reynals, 1957; Martin *et al.*, 1961; Rowson *et al.*, 1961; Tanaka and Southam, 1962, 1965; Stoker, 1963; Salaman and Roe, 1964; Garrett *et al.*, 1993).

Co-carcinogenesis is the phenomenon of additive or synergistic effect of two or more agents leading to cancer. Oncologists described this phenomenon as a complex etiology for cancer – a two-step process of ‘initiation’ and ‘promotion’ of carcinogens to oncogenesis (Southam, 1963; Rous, 1965). In this paper, clinical and epidemiologic evidence will be reviewed that suggests an interaction between viral and chemical carcinogens causes several human cancers. Specifically, the epidemiology of cervical cancer, hepatocellular carcinoma (HCC), and Kaposi’s sarcoma (KS) will be discussed as possible examples of co-carcinogenesis. Postulates for evaluating claims of causality for cancer via co-carcinogenesis will be advanced in the discussion.

## Cervical cancer

Cancer of the uterine cervix is the third most common cancer among women worldwide, behind skin and breast cancers. Incidence of cervical cancer varies by region of the world, ranging from 4.8 per 100 000 in Western Asia to 44.3 in Eastern Africa (See Table 1) (Ferlay *et al.*, 2001). Each year, approximately 465 000 new cases of invasive cervical cancer are diagnosed worldwide, resulting in over 200 000 deaths.

In 1842, Italian investigators reported little to no cervical cancer among Catholic nuns compared to the rest of the Italian female population. Since then, epidemiologists have reported other measures of sexual behavior associated with cervical cancer incidence including marital status, parity, age at first intercourse, numbers of male sex partners, and commercial sex work (Armstrong *et al.*, 1992). These findings led to a search for a sexually transmitted agent as the cause of cervical cancer and theories implicating syphilis, gonorrhea, herpes viruses, and ultimately human papillomaviruses.

In the 1970s, Harald zur Hausen postulated a role for, and then found HPV-DNA in, cervical cancers (zur Hausen *et al.*, 1974, 1975; zur Hausen, 1975, 1976). In the 1980s, his group was the first to isolate HPV-16 and HPV-18 from cervical cancer tissues (Durst *et al.*, 1983; Boshart *et al.*, 1984). Several epidemiologists have subsequently shown highly statistically significant associations between HPV and development of CIN grade 2 or 3 (Koutsky *et al.*, 1992), with persistent CIN 2 (Ho

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**Table 1** Age-adjusted incidence rates (per 100 000) reported for uterine, cervical, and liver cancer cancers by sex for regions of world, 2000, IARC

Region	Cervix (female)	Liver (female)	Liver (male)
World	16.1	5.5	15.0
More developed countries	11.3	2.9	8.7
Less developed countries	18.7	6.8	17.4
East Africa	44.3	6.0	14.4
Middle Africa	25.1	13.0	24.2
North Africa	16.8	2.7	4.9
South Africa	30.3	2.1	6.2
West Africa	20.3	6.2	13.5
Caribbean	35.8	4.2	7.6
Central America	40.3	1.6	2.1
South America	30.9	3.7	4.8
North America	7.9	1.7	4.1
East Asia	6.4	12.7	35.5
Southeast Asia	18.3	5.7	18.3
South Central Asia	26.5	1.4	2.8
West Asia	4.8	2.1	5.6
East Europe	16.8	2.6	5.8
North Europe	9.8	1.4	2.6
South Europe	10.2	3.5	9.8
West Europe	10.4	1.6	5.8
Australia/NZ	7.7	1.2	3.6
Melanesia	43.8	10.2	20.2

Source: Ferlay *et al.* (2001)

*et al.*, 1995) and with development of cervical cancer (Bosch *et al.*, 1992; Ferrera *et al.*, 2000). In 1995, a WHO consensus panel gathered a large body of biologic and epidemiologic data and concluded that at least HPV-16 and HPV-18 infection caused cervical cancer (Anonymous, 1996). HPV can be found in 90–95% of patients with cervical cancer worldwide, most frequently HPV-16 (50%), HPV-18 (12%), HPV-45 (8%), and HPV-31 (5%) (Bosch *et al.*, 1995). However, HPV is a common infection among sexually active young women. For example, there was 43% 3-year incidence of HPV infection among sexually active co-eds at Brown University (Ho *et al.*, 1998). Why do only a few women infected with oncogenic HPV types develop cervical cancer?

Harald zur Hausen was also the first to recognize that HPV was not sufficient for cancer induction, and proposed that HSV-2 and HPV act synergistically to induce cervical cancer (zur Hausen, 1982). Others have postulated a role for HSV-2 infection and cervical cancer, while acknowledging the primary role for HPV (Hildesheim *et al.*, 1991; Daling *et al.*, 1996). For example, Hildesheim *et al.* studied women with invasive cervical cancer in Latin America and compared viral and behavioral characteristics with controls. Compared to women negative for both HPV 16/18 and HSV-2, those positive for HSV-2 alone had a relative risk of 1.2, those positive for HPV16/18 DNA alone had a relative risk of 4.3 (95% CI = 3.0, 6.0), and those positive for both HSV-2 and HPV16/18 had a relative risk of 8.8 (95% CI = 5.9, 13.0), suggesting a possible biological interaction (Hildesheim *et al.*, 1991). Furthermore, HSV-2 was found to be persistent in some cervical

cancer tumors (Frenkel *et al.*, 1972). On the other hand, evidence for HSV-2 infection, measured by antibody testing, can be found in only one-third to one-half of patients with cervical dysplasia or cervical cancer (Mendis *et al.*, 1981; Smith *et al.*, 2002), and even less often in cervical cancer biopsy specimens (Tran-Thanh *et al.*, 2003). What other carcinogens could be involved?

Tar-based vaginal douching was associated with development of cervical cancer by several American investigators. Smith (1931) noted that the use of Lysol® douches was significantly more common among cases than controls. Lombard and Potter (1950) studied women with cervical cancer in Massachusetts; ‘long-continued’ douching with coal-tar derivatives was reported by more cases than controls. In 1967 Rotkin reported the results of a case–control study in which 416 California women with cervical cancer were compared with hospital-based controls matched for age, race, religion, and hospital. Rotkin (1967) found a significant association with Lysol® vaginal douches. This result led to the voluntary removal of Lysol® and other tar-based vaginal douche products from the US market.

Cigarette smoking represents another exposure to a tar-based carcinogen and has been linked to cervical cancer (Hildesheim *et al.*, 2001; Castle *et al.*, 2002). Winkelstein *et al.* (1977) hypothesized that cigarette smoking was a causative factor for cervical cancer. He found a correlation between the age-adjusted incidence rates for cervical cancer and male lung cancer. He noted the results of four case–control studies demonstrating more smoking by women with cancer (Winkelstein, 1977). We have recently updated and expanded Winkelstein’s meta-analyses and found support for his conclusions (Haverkos *et al.*, 2003; Steckley *et al.*, 2003). However, how might tars from cigarettes reach the cervix?

Tobacco smoke contains and delivers over 4000 compounds – some are known carcinogens, such as benzyl (a) pyrenes, polycyclic aromatic compounds and the tobacco specific nitrosamines. One such compound is NNK or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane. The cervical mucus of cigarette-smoking women contains three times the levels of NNK than the cervical mucus of nonsmokers, presumably delivered to the cervix via the blood (Prokopczyk *et al.*, 1997).

Another tar-based compound associated with cervical cancer and possibly the most important on a global scale is derived from carcinogens generated by burning wood in kitchen stoves or ovens. A case–control study was conducted in Honduras to identify cofactors for invasive cervical cancer. Among HPV-positive women, a dose–response relationship was observed for exposure to wood smoke and cervical cancer that persisted in multivariate analysis (Ferrera *et al.*, 2000). In a follow-up study by the same group of investigators, 125 women with cervical intraepithelial neoplasia (CIN I, CIN II or CIN III) in Honduras were compared with 241 age- and clinic-matched controls. Chronic exposure to wood smoke significantly increased the risk of CIN III. It is plausible that chronic inhalation of carcinogens derived from wood smoke could have an

effect on the progression to cervical cancer, similar to that observed for cigarette smoking (Velema *et al.*, 2002).

One can surmise that squamous cell cervical cancer results from a synergistic interaction between oncogenic sexually transmitted agents, principally HPV-16 or HPV-18, and cervical tar exposures. This hypothesis is based on the Rous rabbit model cited above and review of epidemiologic data (Haverkos *et al.*, 2000).

### *Hepatocellular carcinoma*

HCC is one of the major cancers in the world, with approximately 400 000 deaths annually worldwide. Incidence varies greatly by gender and geography (see Table 1). Among males, incidence varies by region ranging from 2.1 per 100 000 in Central America to 35.5 per 100 000 in Eastern Asia. Among females, HCC incidence varies from 1.2 per 100 000 in Australia/New Zealand to 13.0 in Middle Africa (Ferlay *et al.*, 2001). The difference in HCC incidence rates among migrants living in different areas of the world suggests that environmental factors rather than ethnicity or genetics play a major role in etiology. For example, the age-adjusted incidence rates of HCC among Chinese, Japanese, and Filipinos who migrate to Hawaii or Los Angeles are markedly lower than the rates among those who remain in their native country (Chen *et al.*, 1997). Risk factors for HCC include infection with hepatitis B virus (HBV), hepatitis C virus (HCV), and exposure to chemicals, such as aflatoxin exposure, heavy alcohol consumption, inorganic arsenic ingestion, radioactive thorium dioxide exposure, iron overload, oral contraceptives, and anabolic steroid use (Chen *et al.*, 1997; Ferlay *et al.*, 2001; Kew, 2002).

The evidence that HBV infection is causally related to HCC worldwide is strong. HBV infection precedes cancer by many years and HBV DNA is commonly identified in tumor tissues. HCC occurs in geographic areas where HBV is most common (Evans and Mueller, 1990). HBV infection precedes HCC in over 80% of cases in China and Southeast Asia, and about 20% of cases in the USA. Case-control studies have consistently shown a higher prevalence of HBV infection among cases than controls. Furthermore, a related virus (woodchuck hepatitis virus and Peking duck virus) produces HCC in woodchucks and ducks (Evans and Mueller, 1990). HBV vaccine has led to decreases in the incidence of HCC among adolescents in Asia (Chang *et al.*, 1997).

Prospective studies by Palmer Beasley and co-workers provide evidence for a causal role of HBV infection and HCC. Beasley followed 22 707 adult males in government service in Taiwan after testing them for serum hepatitis B virus surface antigen (HBsAg). At 11.5 years of follow-up, 184 of 3434 HBsAg carriers developed HCC compared to 10/19 253 controls (RR = 102.6) (Beasley *et al.*, 1981).

HCV may serve as a cofactor with HBV in some HCC cases and/or play a more significant role in those cases of cirrhosis and HCC not associated with HBV. Egypt

has the highest prevalence of HCV infection in the world; approximately 60% of all HCC cases in Egypt are attributed to HCV infection. In the USA, approximately 9% of HCC cases are attributed to HCV infection (Hassan *et al.*, 2002). In a prospective study of 231 Japanese patients with post-transfusion liver disease not associated with HBV, antibodies to HCV were detected in 95% of 54 patients who developed HCC. The average interval between transfusion and development of HCC was 29 years (Evans and Mueller, 1990).

Multifactorial hypotheses involving virus-chemical interactions to induce HCC are crystalizing. Epidemiologists are reporting synergistic interactions between HBV and aflatoxins in studies of HCC in Africa and Asia, and for hepatitis viruses and heavy alcohol consumption in the USA and Europe. Ming *et al.* conducted two studies in Eastern China. They studied a consecutive series of 181 HCC cases, 80% male. All 181 had one or more serologic markers of HBV infection, six patients (3%) were co-infected with HCV. In the second study, 145 HBsAg positive men were recruited and followed for over 13 years. Exposure to aflatoxin was assessed by measuring a metabolite of aflatoxin, AFM1, by chromatography in pooled urine collected monthly from subjects in 1987. The cumulative dosage of aflatoxin was calculated by multiplying the estimated daily aflatoxin intake by the age (in days) at HCC diagnosis, and the cumulative dose per kilogram body weight based on an assumed weight of 60 kg (Sun *et al.*, 1999; Ming *et al.*, 2002). After 13 years of follow-up, 31 of the 145 men developed HCC; the relative risk for aflatoxin exposure was 3.5 (CI 1.5–8.1) (Ming *et al.*, 2002).

In the USA, investigators in Texas, including Palmer Beasley, conducted a hospital-based case-control study among 115 HCC patients and 230 non-liver cancer controls. Markers of HBV infection, HBsAg and/or antibody to HBV core were found in 24% of patients, and antibodies to HCV in 23%. Multivariate analysis revealed an odds ratio for HCV infection of 15.3 (CI 4.3–54.4), HBsAg of 12.6 (2.5–63.1), heavy alcohol consumption ( $\geq 80$  ml ethanol/day) of 4.5 (1.4–14.8), and diabetes mellitus of 4.3 (1.9–9.9). Significant interactions were noted between alcohol consumption and chronic HBV/HCV infection and between alcohol consumption and diabetes mellitus (Hassan *et al.*, 2002). The authors conclude that a synergistic interaction between heavy alcohol consumption, HBV and/or HCV infection and diabetes mellitus may suggest a common pathway for HCC (Hassan *et al.*, 2002).

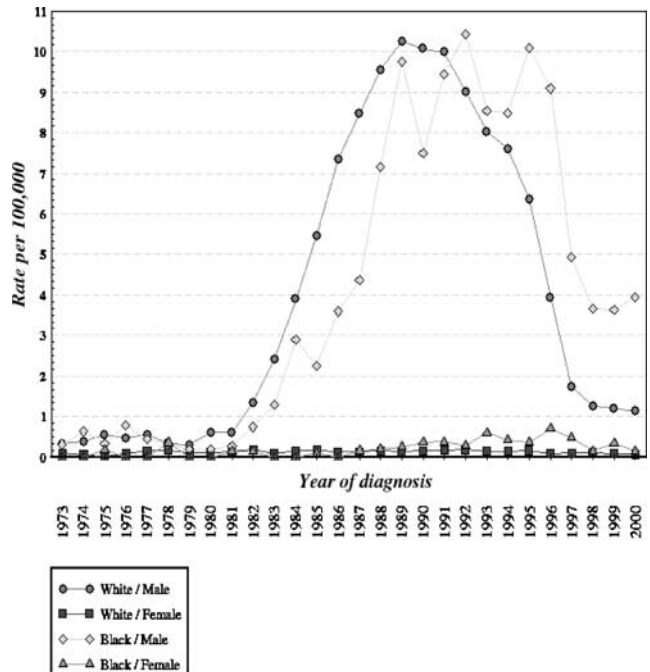
HCC appears to result from the complex interaction of a hepatotoxic viral agents and hepatotoxic chemical carcinogens. Chronic HBV infection is demonstrable in about 80% of HCC cases worldwide, and HCV infection in many of the HBV-negative HCC patients. Exposure to aflatoxins, a food contaminant largely derived from spoiled corn or wheat or groundnuts (peanuts), and heavy alcohol consumption, have long been associated with development of HCC (Sylla *et al.*, 1999; Chen and Chen, 2002; Uetake *et al.*, 2003). These

multifactorial hypotheses result from epidemiologic observations. Understanding the underlying mechanisms of co-carcinogenesis may lead to new strategies to prevent HCC worldwide.

### *Kaposi's sarcoma*

In 1872, Moritz Kaposi described three fatal cases of multiple idiopathic pigmented hemangiosarcoma in elderly men at the University of Vienna. For over 100 years, KS was a rare disease occurring predominantly among elderly Mediterranean and younger African men and boys (Friedman-Kien, 1989). In 1981 the first reports of KS and opportunistic infections among previously healthy gay men and others led to a search for an infectious cause of an underlying immunodeficiency, now known as AIDS (Haverkos and Curran, 1982). In 1983 and 1984, a retrovirus, HIV, was linked to KS and to AIDS (Barre-Sinoussi *et al.*, 1983; Popovic *et al.*, 1984). However, clinicians observed that HIV was not found in most others with KS, that is, African KS, KS in elderly men in the Mediterranean region, or KS associated with organ transplant recipients. The striking epidemiology of KS among AIDS patients, with its sudden increase and then rapid fall, and its occurrence almost exclusively among gay men, led researchers to look for cofactors (Figure 1). The search focused on two areas – factors associated with other forms of KS, and those associated with the gay male lifestyle in the USA and Europe (Haverkos *et al.*, 1985; Beral *et al.*, 1990). The epidemic of HIV-associated KS provided a unique opportunity to decipher the pathogenesis of a malignancy that appears to be multifactorial in origin (Drotman *et al.*, 1995).

In 1994 Chang, Moore, and co-workers reported DNA sequences of a new herpes virus, HHV-8, in KS tissues (Chang *et al.*, 1994). HHV-8 has been documented to occur in greater than 95% of KS patients of all forms, suggesting that HHV-8 is a necessary etiologic agent (Moore and Chang, 1995; Whitby *et al.*, 1995). The prevalence of HHV-8 antibodies varies widely across populations with good correlation between seroprevalence and incidence of KS. The prevalence of antibodies to HHV-8 is common among gay men in the USA (15–60%), but infrequent among heterosexuals (0–9%) (Osmond *et al.*, 2002). Gay men with KS are more likely to have HHV-8 DNA in peripheral blood than gay men without KS (35 *versus* 6%) (Cannon *et al.*, 2003), found that the prevalence of HHV-8 in 1978 and 1979 was 26.5% among gay men in one San Francisco cohort, and that its prevalence did not change through 1996 (Osmond *et al.*, 2002). On the other hand, the rapid increase in the incidence of HIV-associated KS in the early 1980s and its decline in the United States prior to the era of highly active antiretroviral therapy suggests that HHV-8 alone does not cause KS. If HHV-8 and HIV jointly cause KS, then one would expect KS to 'spread' to heterosexuals dually infected with HIV and HHV-8, which has not yet been observed. Furthermore, the decline in KS prior to HAART has not been explained (see Figure 1).



**Figure 1** SEER incidence and age-adjusted rates of KS, nine registries, 1973–2000. Surveillance, Epidemiology, and End Results (SEER) Program ([www.seer.cancer.gov](http://www.seer.cancer.gov)) SEER\*Stat Database: Incidence – SEER 9 Regs Public-Use, Nov 2002 Sub (1973–2000), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2003, based on the November 2002 submission

The epidemic of HIV-associated KS provides a unique opportunity to decipher the pathogenesis of a malignancy that appears to be multifactorial in origin. Use of nitrite inhalants should be considered another carcinogen that is consistent with the unique epidemiology of HIV-associated KS. Gay men used this aphrodisiac more commonly than did heterosexuals prior to the AIDS epidemic and use has been declining due to its now illegal status, and the increased availability of other vasodilators (Haverkos *et al.*, 1994). Some, but not all, epidemiologic studies have shown a statistical association between the development of KS among gay men with AIDS and the use of large quantities of nitrite inhalants when compared to gay men with AIDS, but without KS (Haverkos and Drotman, 1995). Anecdotal reports of increased frequency of AIDS-related KS on the chest and face, especially the nose, and in the lungs are consistent with the body areas most heavily exposed to nitrite vapors when inhaled. Finally, plausible mechanisms of actions have been proposed. Nitrites act on blood vessels, the presumed site of KS, and their metabolites, cholesteryl nitrite and nitrosamines, are known mutagens (Mirvish *et al.*, 1993).

The African endemic form of KS was first described in 1914 and occurs predominantly in black males 25–40 years of age but also children at a mean age of 3 years (Friedman-Kien, 1989). The introduction of HIV infection led to KS becoming the most common cancer in

men in several areas of Africa (Dal Maso *et al.*, 2001). John Ziegler and others have studied risk factors for KS among both HIV-seropositive and HIV-seronegative people in Uganda. Ziegler proposes that KS results from the activation of latent HHV-8 by immune suppression or inflammation to an oncogenic, lytic state in some endothelial cells. Walking barefoot among volcanic soils, whereby aluminosilicates in the soil, aided by quartz abrasions, enter sweat glands and pores of the feet, and thus promote carcinogenesis (Ziegler, 1993; Ziegler *et al.*, 1997, 2001).

One might propose that the AIDS-related form of KS in the USA and Europe results from a complex interaction of HHV-8, HIV, and nitrite inhalant abuse (Haverkos, 1996). In Africa HHV-8 may be reactivated by HIV and/or by carcinogens or abrasives in the soil or other environmental sources (Ziegler *et al.*, 2001).

## Discussion

Establishing the cause of a cancer has major clinical, public health and scientific benefits. Several epidemiologists, among them Alfred S Evans (1917–1999), recognized several problems with the ‘gold standard’ of causation, the Henle-Koch postulates, establishing that viruses cause cancer. The problems included the long incubation period between infection and onset of cancer, the frequency of infection in relation to the rarity of the cancer, the presence of cofactors, the complexity and multistage process of most cancers, and the inability to reproduce human cancers in animal models (Evans and Mueller, 1990). Evans concluded that the cause of both infectious and noninfectious diseases, including cancer, involved a complex interplay of agents, environmental, and host factors. Hypotheses involving viruses and chemicals for the etiology of selected cancers can be generated (see Table 2).

Current research concepts of cancer causation have, for a large part, focused on the establishment of the causal role of a single viral or chemical carcinogen in the production of disease. These approaches to causative proof have concentrated on two ingredients: the suspected factor and the genetics of the human host. In 1982, Evans suggested that clinical scientists focus on ‘clinical promotion factors’ as a third ingredient. Evans refers to a short story by O Henry in which a poor girl has only a piece of beef. She meets a young woman with a potato. Together they join the ingredients to make a stew. After tasting it the two women recognize that it is not a stew without an onion. The rest of the story concerns the search for ‘a third ingredient,’ the title of the short story (Evans, 1982). The interactions between an oncogenic virus and a susceptible host involve a series of responses that range from asymptomatic to invasive/metastatic cancers. The factors that promote clinical manifestation of disease are poorly understood. Co-carcinogenesis is an ‘old’ concept in cancer research that needs to be revived.

One of the most perplexing questions with respect to viral and chemical carcinogenesis is why, when many individuals have extensive exposures to several viral and chemical carcinogens, only a relatively few develop site specific cancers. Timing of exposures and genetic predisposition to disease may be additional etiologic factors. Although Reye’s syndrome is not a cancer, understanding its epidemiology and pathogenesis may provide useful insights. Reye’s syndrome is reported to result in children when aspirin therapy is given during a prodromal stage of varicella zoster virus or influenza B virus infection. Rowe *et al.* (1988) evaluated four consecutive patients referred for intensive care of Reye’s syndrome. Two of the children had enzymatic defects of fatty acid oxidation, two had partial deficiencies of ornithine transcarbamoylase. Genetic or acquired enzyme abnormalities may affect the metabolism of salicylates at a critical point in a viral infection leading to increased levels of hepato-/neurotoxins characteristic

**Table 2** Hypotheses – etiology of selected human cancers

<i>Infectious agent</i>	<i>Co-carcinogens</i>	<i>Cancer</i>	<i>Reference</i>
HPV-16, 18, etc	Tar-based vaginal douches, tobacco, cooking over burning wood, HSV-2	Cervical cancer	Ferrera <i>et al.</i> (2000), Haverkos <i>et al.</i> (2003) and zur Hausen <i>et al.</i> (multiple)
HBV	Dietary aflatoxins	Hepatocellular carcinoma	Beasley <i>et al.</i> (1981), Beers (1973) and Ming <i>et al.</i> (2002)
HBV/HCV	Alcohol	Hepatocellular carcinoma	Hassan <i>et al.</i> (2002)
HHV-8	HIV, nitrite inhalants	HIV-related Kaposi’s sarcoma	Chang <i>et al.</i> (1994) and Haverkos (1996)
HHV-8	Immunosuppressants, exposure to water and/or volcanic soils, dietary nitrosamines	Kaposi’s sarcoma in Uganda	Ziegler (multiple)
EBV	Malaria, nitrosamines	Burkitt’s lymphoma	Evans and Mueller (1990)
EBV	PCBs, pesticides, hair dyes	Non-Hodgkin’s lymphoma	Evans and Mueller (1990), Holly <i>et al.</i> (1998) and Rothman <i>et al.</i> (1997)
HPV	Alcohol, tobacco	Oral cancer	Day <i>et al.</i> (1994) Gillison and Shah (2003)
SV40	Asbestos, tobacco	Mesothelioma	Carbone <i>et al.</i> (2003)

EBV = Epstein–Barr virus; HBV = hepatitis B virus; HCV = hepatitis C virus; HHV-8 = human herpes virus 8; HIV = human immunodeficiency virus; HPV = human papillomavirus (several types); HSV-2 = herpes simplex virus 2; PCBs = polychlorinated biphenyls; SV40 = Simian virus 40

of Reye's syndrome. Similar enzymatic factors may contribute to the metabolism of carcinogens in genetically predisposed individuals.

If cancer results from stepwise multifactorial exposures or a synergistic interaction of co-carcinogens, then Koch's postulates will never be satisfied because they only apply to a single infectious agent. A new set of criteria for evaluating claims of causality for diseases that are multifactorial in etiology are needed. Robert Root-Bernstein, Michigan State University, has proposed causality criteria for autoimmune disorders (Root-Bernstein, 1993). In order to continue Root-Bernstein's discussion and attempt to apply them to cancer etiology, one might propose the following postulates:

- (1) Two or more infectious or carcinogenic agents will be active simultaneously.
- (2) Exposure to only one of the agents may lead to a different disease, such as a known precursor condition.
- (3) These multiple agents will be isolatable or identifiable individually.
- (4) No single agent will be capable of inducing the cancer by itself when introduced into healthy humans or animals, or will do so only rarely and under limited circumstances.
- (5) The cancer may be reproduced in an animal model by means of an appropriate combination of the putative causative agents.
- (6) The incidence rate of the cancer will vary directly with the quantity and prevalence of exposure to each of the putative agents in a population, and thus will decrease with the removal of exposure to one or more of those agents.

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