Commentary

Multifactoral etiology of human liver cancer

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Introduction

Liver cancer is one of the most prevalent forms of cancer in the world. Hepatitis B virus (HBV)* is considered to be a major etiological factor (1,2), and an intervention strategy based on vaccination has been formulated (3). Evidence from epidemiological studies has also indicated that environmental contaminants, such as mycotoxins, may either in combination with HBV or independently be important etiological factors in the pathogenesis of primary hepatocellular carcinoma (PHC). This commentary is a brief review of the epidemiological and laboratory data that suggest interplay of viral and chemical factors in the multifactorial etiology and pathology of PHC. Areas of uncertainties and future experimentation are identified and a hypothesis of liver carcinogenesis is proposed.

Multifactoral etiology

Epidemiology

The incidence of PHC is highest in Africa and Asia (4). Attention has been focused on HBV because (i) prospective epidemiological studies have shown a high incidence of PHC in these HBV-endemic areas (5–7); (ii) of the clinical observation that the majority of the patients with PHC are carriers of hepatitis B surface antigen (HBsAg) and have chronic active hepatitis (CAH) (2,6,7); and (iii) recently, integrated HBV sequences have been found in the hepatocyte genome in patients with chronic hepatitis, hepatocellular carcinoma, and in HBV carriers (8–10).

PHC is less common and occurs at an older age in urban rather than in rural populations. HBV infection at an early age in the rural areas is considered to be the explanation for these observations. However, recent evidence suggests that other environmental factors are important. For example, a case control study of PHC patients of whom all were born and reared in rural areas but half had then moved to an urban setting was recently conducted by Kew et al. (11). They concluded that HBV status, which was similar in both groups, was not responsible for the differences in incidence and age at onset of PHC in rural and urban populations. Sun et al. (12) have reached a similar conclusion based on data from China.

The additional environmental factors most likely to increase the risk of PHC include exposure to aflatoxin B₁ (AFB₁) (13–16), consumption of alcoholic beverages (17–19) or contaminated water (20), occupation (21), tobacco smoking (22–24), and androgen therapy (25). In the People’s Republic of China, the correlation between PHC incidence and estimated dietary mycotoxin intake is statistically higher than the correlation between PHC incidence and the geographic distribution of HBV infection (26). Whereas the incidence of HBV infection does not vary among people living in the low- and the high-altitude areas of Kenya, both the incidence of food contamination by AFB₁ produced by Aspergillusflavus and the incidence of PHC are higher in the low-lying regions (14).

Consumption of alcoholic beverages has long been considered to be a major etiological factor in the pathogenesis of PHC in Western countries (17,27,28). A recent case control study has also suggested that drinking of alcoholic beverages may be an important factor for PHC in the Philippines (29). Individuals consuming daily more than both 21 g of alcohol and an estimated >4 μg of AFB₁ in contaminated food, primarily cassava and corn, had a 35-fold increased relative risk of PHC. The effects of alcohol intake and dietary mycotoxin intake were synergistic (29). The results suggest that the role of alcoholic beverages, both free of and contaminated with mycotoxins, should be further examined in other Asian countries and in Africa.

Progress has been made in identifying chemical carcinogens in human body fluids and tissue samples. Both AFB₁ metabolites (e.g., aflatoxin M₁ [AFM₁] [30–33], and nucleic acid repair products (34) can now be measured in body fluids by both immunological and chemical methods, and these products have been detected in urine samples from individuals ingesting contaminated foods. Urinary excretion of AFM₁ from local inhabitants in areas of high PHC prevalence in the People’s Republic of China was found to be significantly elevated, up to 40-fold or more, over that found in people in areas of lower PHC incidence (31,32). During the wet season, the amount of AFM₁ excreted in the urine may increase 100-fold over that excreted in the dry season. By direct measurement of AFB₁ in contaminated alcoholic beverages and the urinary excretion of AFM₁ from people who drank the beverages, the AFB₁ to AFM₁ conversion ratio is calculated to be 1.5% (33). Estimation on the basis of these data has shown that AFB₁ intake in ~10% of adults living in the high-incidence area exceeds 1 mg per year. The primary source of contamination was found to be corn and rice; the second is local undistilled alcoholic beverage obtained from these grains. Since males generally eat more food and also drink more alcoholic beverages than females, both the 3-fold or more male preponderance of PHC and the age peak in the fourth and fifth decades of life in areas of prevalence — two facts difficult to explain by viral etiology alone — can be at least partly attributed to AFB₁ and/or alcohol exposure.
Food samples collected in Murang'a District, Kenya, are known to be contaminated with AFB1, and one of its putative nucleic acid repair products, 2,3-dihydro-2-(7'-guanyl)-3-hydroxyaflatoxin B1, has been detected in human urine collected in this region of high cancer risk (34). Recent findings from this ongoing biochemical epidemiological investigation have also shown a seasonal variation in that urinary adducts are found more frequently during the months of high contamination of food by AFB1 (Autrup.H., Shamsuddin.A.K., Vahakangus.K., Oravec.L., Mann,D.L. and Harris,C.C., unpublished results). The presence of this adduct in the urine is an indication of exposure of AFB1, of its metabolic activation, and of interaction between the ultimate carcinogenic form of AFB1, and cellular nucleic acids in vivo and further supports the hypothesis that AFB1 may play an important role in the etiology of human liver cancer.

Measurement of AFB1-modified DNA by enzyme radioimmunoassays (35,36) in liver samples from males and females will be of particular interest in regard to the issue of male preponderance of PHC. It will also allow the critical evaluation of the quantitative relationship, if any, between adduct levels and risk of developing PHC. Other approaches, e.g., measures of serum interferon levels, histocompatibility membrane antigens, in situ hybridization of HBV nucleic acid sequences, and restriction enzyme fragment length DNA polymorphism, may also provide both clues to identify individuals at high risk for PHC and additional insights into the pathogenesis of human PHC.

Animal models
Liver carcinogenesis, including the identification of putative precursor lesions of PHC, has been extensively studied in experimental animals (see reviews 37 – 39). Numerous carcinogens from different chemical classes, e.g., N-nitrosamines and aromatic amines, cause PHC in several animal species. One of the most remarkable features of the putativepreneoplastic cells found in the carcinogen-induced liver nodules is their resistance to different types of cytotoxic agents. Regardless of the model system of liver carcinogenesis used to produce the nodules, a set of common phenotypic changes, perhaps an adaptive response analogous in principle to that found in the heat shock response of Drosophila (40), occurs in the resistant nodular cells, including increased levels of glutathione, epoxide hydrolase, and xenobiotic conjugating enzymes and decreased amounts of cytochrome P450. These phenotypic changes most likely alter the metabolic balance between activation and deactivation of procarcinogens and other xenobiotics, and make the cell more resistant to injury caused by electrophilic reactants and other cytotoxic metabolites.

AFB1 is one of the most potent hepatocarcinogens known in experimental animals (14,16). AFB1 also produces cancers in other tissues, including bile duct, pancreas and bone in monkeys (41), and in rats, a shift in cancer incidence from PHC to intestinal carcinomas occurs in vitamin A deficiency (42). Other nutritional factors, including choline and methionine deficiency, have striking effects on PHC incidence (43 – 45). Because the organ specificity of chemical carcinogens varies among animal species and is influenced by both environmental and host factors, we suggest that AFB1 and other suspected chemical hepatocarcinogens in humans may also contribute to the etiology of other types of cancer in geographic areas contaminated by these compounds.

In the area of high incidence of human PHC, only the duck (Anas domesticus) among domestic animals was found to have a high incidence of liver tumors (46). Duck hepatoma deserves special attention due to the following facts: (i) its prevalence correlates with the increasing incidence of human PHC in the different regions of Qidong (46); (ii) ducks are highly susceptible to the carcinogenic effects of food contaminated by mycotoxins, including AFB1 (47); and (iii) HBV-like virus has been found in the ducks (48 – 50). Similar particles have been observed in hepatocytes and hepatoma cells from ducks with viremia (51). The potential interactive effects of this viremia and food contaminated by aflatoxins is suggested by an analysis of the data reported by Qian et al. (47). When ducks in an area where flocks are commonly infected with the HBV-like virus were fed aflatoxin-contaminated corn, 18% (25 in 140 animals) developed hepatomas whereas no hepatomas were found in the 40 ducks fed non-contaminated corn. These experiments need to be repeated in virus-infected versus virus-free flocks.

In addition to the duck, HBV-like viruses have also been detected in the eastern woodchuck (Marmota monax) and the ground squirrel (Spermophilus beecheyi) (49). Chronic infection by HBV-like virus appears to be causally related to the subsequent development of liver cancer in the woodchuck (52).

Pathophysiology of human hepatocellular carcinogenesis
PHC most frequently arises in patients with CAH in which dynamic pathohistological lesions of hepatic cell necrosis, proliferation, dysplasia accompanied by inflammation and frequently cirrhosis are observed (see review, 53). In the non-tumorous hepatocytes, HBsAg and HB core antigen (HBcAg) can be found by immunocytochemical techniques. Dysplastic and neoplastic hepatocytes may express HBsAg and alpha-fetoprotein but are rarely positive for HBcAg (54 – 56). Cultured and xenotransplanted PHC cell lines may also produce HBsAg and alpha-fetoprotein but HBcAg has not been detected in them (57 – 60). Alpha-fetoprotein is also found in the serum of PHC patients (see review, 61). The incidence of PHC among CAH patients having a progressive, fluctuating pattern of elevated alpha-fetoprotein may reach 2 – 5% per annum, a risk of PHC that is much higher than that in 'healthy' HBV carriers (61). Hepatocellular cytotoxicity is considered to be caused by hemopoietic cell-mediated cytotoxic response to HBsAg (62,63) and/or HBcAg (64). In addition, data from experiments in which HBcAg gene has been transfected into human epithelial cells suggest that large quantities of intracellular HBcAg are cytotoxic (60). During CAH, the surviving hepatocytes are stimulated to regenerate the liver. The complex of endogenous and exogenous mediators thought to be involved in the induction and maintenance of the regeneration of the hepatic response, including hormones, growth factors and nutritional status, in the surviving hepatocytes has been extensively investigated in the partial hepatectomy model in the rat (65), but the extrapolation of these findings to humans is uncertain.

Hypothesis of hepatocellular carcinogenesis
We propose that the preneoplastic and neoplastic hepatocytes attain resistance to mediators of cell destruction (thus having a survival advantage) and undergo proliferation during the repetitive cycles of the regenerative phase of CAH (Figure 1). This hypothesis of selective clonal cell expansion is consistent...
with concepts derived from studies using animal models of liver carcinogenesis (53,66). Following an initiating dose of a genotoxic agent, a 'programmed' response is triggered, producing a cellular phenotype that has a survival-growth advantage, including resistance to the cytotoxicity of environmental chemicals and/or their metabolites and to the cytopathology related to HBV infection. This clone(s) of resistant cells expands in response to an endogenous proliferative factor(s) following partial hepatectomy and/or cell destruction of the nonresistant, normal cells by chemical and microbial toxins and by a host-mediated cytopathic response. In this context, CAH can be considered as a 'viral partial hepatectomy'. The contributions of cytopathicity caused by mycotoxins (e.g., aflatoxins from Aspergillus and trichothecene toxins from Fusarium) and/or alcoholic beverages and of subsequent liver regeneration may also be important, especially in patients with certain nutritional deficiencies.

What is the mechanism(s) for producing a resistant phenotype in CAH? Several possibilities seem plausible (Table I). The first general type of mechanism would be genetic control of the HBV genome integrated into PHC cells and their precursor cells. Methylation of the integrated HBV genome by the resistant hepatocytes could inhibit transcription of HBV mRNA and expression of HBV gene products, including those on the cell surface and those (e.g., HBCAg) that may be cytotoxic (60). This mechanism is compatible with data showing an inverse relationship between methylation level and expression of certain viral and nonviral genes (67, 68). Recent studies using Alexander hepatocellular carcinoma cells and HBCAg gene transfected human cells have also shown that HBCAg expression is partly controlled by methylation (60,69). Rearrangements of and mutations in the integrated HBV genome caused by exposure to chemical carcinogens are other genetic mechanisms that warrant investigation. PHC cells arising by these mechanisms would contain HBV genomic sequences in their DNA, but expression of the integrated HBV genome, especially the HBCAg gene, would be restricted, a predication that is consistent with the immunocytochemical observations demonstrating a reduction in HBsAg and HBCAg in dysplastic and neoplastic hepatocytes.

Because not all PHC contain integrated HBV sequences, we also propose a second general mechanism in which resistance to the cytopathological effects of HBV infection would be due to 'non-infectivity' of and/or restriction of proviral replication in the carcinogen-altered hepatocytes. For example, the presumed membrane receptor for HBV could be

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**Table I.** Putative selective clonal expansion factors in human liver carcinogenesis

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<thead>
<tr>
<th>Cell destruction mechanisms in non-neoplastic cells</th>
<th>Cell resistance mechanisms in neoplastic and neoplastic cells</th>
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<tbody>
<tr>
<td>Lymphocyte-mediated cytotoxicity</td>
<td>Methylation of hepatitis B virus genome (e.g., HBCAg gene)</td>
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<tr>
<td>Intracellular cytotoxicity by hepatitis B core antigen</td>
<td>Rearrangement of hepatitis B genome</td>
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<tr>
<td>Environmental chemical 'cytotoxins'</td>
<td>Mutations caused by chemical carcinogens in the integrated hepatitis B virus genome</td>
</tr>
<tr>
<td>Mycotoxins (e.g., aflatoxins, trichothecenes)</td>
<td>Non-infectivity of the hepatocytes by hepatitis B virus (e.g., receptor modification)</td>
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<tr>
<td>Alcoholic beverages</td>
<td>Restricted replication of proviral hepatitis B virus</td>
</tr>
<tr>
<td>Others</td>
<td>Increased detoxification of environmental chemical 'cytotoxins'</td>
</tr>
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*Nutritional, hormonal and inherited host factors may play important roles.
blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be envisioned as mechanisms. One would also predict that at least HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms. In addition, restriction by a putative repressor protein of HBV replication and/or integration into the host cell DNA could be blocked by HBsAg or modified by other mechanisms.
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