The multifactorial etiology and multistage pathogenesis of HHC have fascinated a wide spectrum of cancer researchers for decades. The discovery of HBV and the development of immunological reagents for its detection allowed cancer virologists and epidemiologists to conduct definitive case-control studies demonstrating a strikingly high relative risk of HHC in HBV carriers with chronic active hepatitis (1). Individuals infected with HBV who mount a curative immune response and/or do not develop chronic active hepatitis are at a much lower risk. These findings indicate that cell proliferation and/or inflammatory response associated with chronic active hepatitis are the critical factors responsible for the increased probability of neoplastic transformation of the precursor cells of HHC. Insertional mutagenesis by HBV integration into the human genome also has been suggested as a mechanism to inactivate tumor suppressor genes and to activate protooncogenes (2). However, when compared with the high frequency of viral integration in or near the myc genes in the woodchuck model of liver carcinogenesis (3), HBV integrates at random sites in HHC (4). Although insertional mutagenesis may be sufficient to initiate liver carcinogenesis, it is not necessary, because not all HHC contain integrated HBV sequences (4), and hepatitis C virus, which does not integrate, also increases the risk of HHC (5).

Consumption of alcoholic beverages has long been considered to be a major etiological factor in the pathogenesis of HHC in Western countries (6). Interestingly, the contribution of ethanol and its major toxic metabolite, acetaldehyde, in the carcinogenicity of these beverages continues to be debated. Other HHC risk factors include tobacco smoking, consumption of diets low in selenium, and androgen therapy.

AFB is considered to be a significant etiological factor in certain geographic areas (e.g., southern Africa and Asia) where food contaminated by this mycotoxin produced by Aspergillus flavus is consumed (7). Epidemiological studies conducted in the 1970s and 1980s provided statistical evidence that dietary consumption of AFB was positively correlated with incidence of HHC and indicated a synergistic interaction with either consumption of alcoholic beverages (8) or chronic active viral hepatitis (9-11). These results stimulated a molecular epidemiology study of the HHC risk factors involving a multidisciplinary collaboration between cancer epidemiologists and laboratory researchers. This landmark cohort study (12) of 18,244 people utilized “instruments” from both analytical epidemiology (i.e., a structural questionnaire, in-person interviews, and the use of a cancer registry) and the laboratory (i.e., analytical chemical analysis of food and biomonitoring of serum and urine collected from the subjects for HBV and AFB by immunological and biochemical techniques). The results from this prospective study provide convincing evidence that AFB has an etiological role in hepatocellular carcinogenesis and indicate a possible synergism between the risk factors of HBV and AFB.

Qian et al. (13) report in this issue of Cancer Epidemiology, Biomarkers and Prevention a follow-up study of this cohort in which a nested case-control analysis shows statistically significant associations among the presence of AFB and its metabolites in urine specimens, serum HBV surface antigen positivity, and HHC risk. The increased number of HHC cases and controls and the molecular dosimetry of urinary AFB-N7-guanine adduct in this report strengthens the conclusions from the initial study. The presence of the promutagenic AFB-N7-guanine adduct is further evidence that AFB has been activated into its ultimate carcinogenic metabolite, AFB 8,9-oxide (7). Human hepatocytes in vitro can enzymatically activate AFB to its 8,9-oxide (14) and the interindividual variation in forming the AFB-N7-guanine adduct may be 10-fold or greater (15). Since several isoforms of cytochrome P-450 enzymes can activate AFB (16), the interpretation of pharmacokinetics data and its use in risk assessment will be complicated. The mutational spectrum of AFB has been established in experimental systems and G:C to A:T transversions are the most common base substitutions. The high frequency of G:C to A:T transversion at codon 249 (AGG->AGT) of the p53 tumor suppressor gene in HHC from areas of China and Mozambique with a high incidence of liver cancer (reviewed in 17) could be due to the high mutability of the third base of codon 249 by AFB and/or a selective growth advantage of hepatocyte clones carrying this specific mutant in liver chronically infected by HBV. Cerrutti et al. (18) have found that the third base in codon 249 in a human liver cell line exposed to AFB is preferentially mutated. Other p53 codons show a lower frequency of G:C to T:A, G:C to A:T and G:C to C:G mutations, which indicates that both preferential mutability and clonal selection are involved in HHC. This highly sensitive and specific genotypic mutation assay also can be used to determine the p53 mutation load in nontumorous liver tissue from donors in which urinary AFB and/or macromolecular-AFB adducts have been detected.

Only a portion of the puzzle of human liver carcinogenesis has been solved (i.e., the longstanding debate between the virology and chemical carcinogenesis communities concerning the etiological role of AFB). Attention can

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2 The abbreviations used are: HHC, human hepatocellular carcinoma; HBV, hepatitis B virus; AFB, aflatoxin B1.
now be devoted to more current questions. For example, what are the acquired and inherited host factors that determine which individuals infected with HBV will develop chronic active hepatitis? Will HBV oncproteins bind to and inactivate cellular proteins encoded by tumor suppressor genes such as p53 or Rb? How much of the HHC risk is attributable to dietary AFB in the high incidence geographic areas of HHC? Will alcoholic beverages, AFB, tobacco smoke and HBV have interactive effects on HHC risk? Does dietary exposure to AFB increase the probability of the development of chronic active hepatitis in people exposed to HBV? Does AFB cause mutations in HBV genome that increase its pathogenic effects? Does CAH alter AFB metabolism? In geographic areas where AFB is not considered an HHC risk factor, are there other environmental chemical carcinogens with positive interactive effects on chronic HBV or hepatitis C virus infections? Will combined HBV and hepatitis C virus infections have additive or synergistic interactions in the risk of HHC? While these and other pieces are being properly placed to complete the HHC puzzle, we already know enough to institute preventative measures. HHC causes at least 200,000 deaths world-wide each year and in some regions, such as Qidong, People's Republic of China, this disease causes 10% of all deaths. As a major public health problem, both large scale HBV vaccination programs and the limitation of AFB exposure in the food supply are important strategies to decrease the incidence of this disease. Vaccines for HBV are well developed and vaccination programs are being implemented in many areas of Africa and Asia. Primary prevention measures for aflatoxin exposures through improved storage of food grains can also be instituted. Secondary prevention measures using chemopreventive agents that block the activation and enhance the detoxification of AFB also are technically feasible. Although the anticipated reduction in HHC incidence due to these preventive measures will require several decades, decreases in the frequency of chronic active hepatitis, urinary AFB positivity, and p53 mutation load may be informative intermediate endpoints to evaluate the putative success of these measures.

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