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Molecular Dosimetry of Aflatoxin Exposure: Contribution to Understanding the Multifactorial Etiopathogenesis of Primary Hepatocellular Carcinoma with Particular Reference to Hepatitis B Virus

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Aflatoxin exposure and hepatitis B virus infection have been implicated as major risk factors for primary hepatocellular carcinoma (PHC) in high-incidence regions of the world. Investigations using the assay of aflatoxin bound to peripheral blood albumin have shown that exposure can occur throughout the life span of the individual, including during the perinatal period, in high-incidence areas such as The Gambia, Senegal, Kenya, and The People's Republic of China. The possibility of measuring aflatoxin exposure at the individual level permits an investigation of the putative mechanisms of interaction of this carcinogen with HBV in the etiopathogenesis of PHC. Animal models, e.g., Pekin duck and HBV-transgenic mice, have also been used to study these questions, and the available data are reviewed.

Introduction

Primary hepatocellular carcinoma (PHC) is one of the most common cancers worldwide, with a high mortality among cases. Hepatitis B virus (HBV) infection and aflatoxin exposure have been identified as major risk factors in the high-incidence areas of Africa and Southeast Asia (*1*), and programs to vaccinate against HBV have been implemented in various countries, including The Gambia, with the perspective of observing a reduction in the incidence of this cancer in those areas (*2*). The carcinogenicity of aflatoxin B_1 (AFB_1) in humans has been difficult to assess, despite the wealth of research on this compound since its chemical identification in the early 1960s (*3*). Although the carcinogenicity in animals, including primates, is well established, the epidemiological data for risk of PHC are inevitably less conclusive. However, the data were sufficiently strong for an IARC Working Group (*4*) to conclude that there was "sufficient evidence of carcinogenicity in humans" (classified Group 1). The epidemiological data are mainly confined to ecological correlation studies (*1*), and the limitation of this type of study design is well recognized. Studies both in Africa (*5*) and the People's Republic of China (*6*) controlling for HBV carrier rates have suggested an important contribution of aflatoxin to PHC rates. More recently, a large correlation study in China reported no association between PHC and exposure to aflatoxin, as determined by detection of aflatoxin metabolites in the urine (*7*); the conclusions of this study have been questioned, mainly because of the method used to assess aflatoxin exposure (*8*). Recent data on liver tumors from China (*9*) and southern Africa (*10*) have added a further dimension to the discussion in suggesting that the high frequency of a specific point mutation (G to T transversion) in codon 249 of the *p53* tumor-suppressor gene in PHC from these areas is related to AFB_1 exposure, which is reportedly high in these populations. This mutation is apparently not related to HBV infection (*11,12*).

In all the studies described above, two limitations are evident. First, there is a notable absence of studies considering exposure at the individual level (e.g., case–control and cohort studies), because of an absence of appropriate exposure markers at that level, and second, there is a difficulty in assessing an independent risk for aflatoxin in the presence of a strong, specific association between HBV and PHC. The latter difficulty is exacerbated by the lack of understanding at a mechanistic level of the possible nature of the interaction between aflatoxin and HBV infection in the etiopathogenesis of PHC. It is still not evident if the development of PHC in HBV chronic carriers is the result of specific genetic changes attributable to HBV or indirect (nonspecific) mechanisms resulting from recurrent cell death

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and proliferation. Research to overcome the above limitations will be valuable in providing data to accurately assess the risk of developing this cancer from exposure to aflatoxin and would also contribute to effective planning of intervention to reduce aflatoxin exposure.

Recently, a number of methods to quantitate exposure at the individual level have been developed (*13,14*). Specifically, the assay of aflatoxin bound to peripheral blood albumin (*15–19*) has been shown to be suitable for use in epidemiological studies of aflatoxin and potential applications of the method have been described (*18,20,21*). In addition, several animal models are available to study the possible mechanism of interaction between HBV and aflatoxin.

This paper describes the information that has been derived about the level and duration of human exposure to aflatoxin using the measurement of the aflatoxin–albumin adduct. These data are presented in relation to the possible stages and mechanisms of interaction with HBV infection and are discussed in the light of recent data from animal model and field studies.

Aflatoxin–Albumin Adducts in Human Sera

The assay that has been used in our laboratory to assay aflatoxin–albumin adducts has been described in detail (*16–18*). Briefly, albumin is precipitated from serum or plasma, quantitated, and a known amount is enzymatically hydrolyzed. The aflatoxin residues from the hydrolysis are purified either by Sep-pak C18 cartridges before quantification in an ELISA or by an additional step on an aflatoxin antibody affinity column before analysis of a major adduct, AFB_1–lysine, by HPLC with fluorescence detection. Attention has been drawn to the fact that hydrolysis will yield a number of different aflatoxin residues and that the quantitation in immunoassay will depend on the affinity of the antibody for each of these residues (*22*). A similar point is relevant to quantitation of urinary aflatoxin metabolites (*22*). In the approach which we have used, *a*) the antibody used in the immunoassay appears to recognize the hydrolysis products with the same affinity as the AFB_1–lysine standard (*18*) and *b*) confirmation of immunoassay results by HPLC-fluorescence has demonstrated a good correlation between the measures of total hydrolysis products and AFB_1–lysine (*16*).

Sera from a number of different human populations have been assayed in a number of separate studies, and a summary of these data is given in Table 1. Some observations on these collated data are discussed below.

First, exposure is significantly higher in both West and East Africa and the Guangxi region of the People's Republic of China than in Thailand, while in Europe aflatoxin adducts in all sera have to date been nondetectable. The lower level of adducts in Thailand may reflect the fact that, although aflatoxin is present in Thai foods (*24*), the levels in rice, the staple for most of the country, are rarely elevated. This contrasts with The Gambia, Senegal, and Kenya, where the staple foods, groundnuts or maize, are highly susceptible to aflatoxin contamination.

High levels (up to 100×10^6 nucleotides) of aflatoxin–DNA adducts have been reported in human liver tissues from individuals from Europe (*25*), similar to those seen in tissues from Taiwan (*26*). The origin of this exposure in Europe is unknown,

Table 1. Aflatoxin–albumin adducts in human sera.[a]

	No. of subjects with different adduct levels, pg AFB_1–lysine eq./mg albumin					
Country (*n*)	<5[b]	5–25	26–50	51–75	76–100	>100
The Gambia						
May (323)	7	53	76	49	40	98
November (67)	0	39	13	7	3	5
Senegal (29)	0	20	6	2	1	0
Kenya (91)	48	26	5	1	5	6
China						
Guangxi (93)	28	35	13	6	2	9
Shandong (69)	69	0	0	0	0	0
Thailand (84)	73	10	1	0	0	0
France (44)	44	0	0	0	0	0
Poland (30)	30	0	0	0	0	0

AFB_1, aflatoxin B_1.

[a]Some of these data are taken from Wild et al. (*16*). The data were obtained in collaboration with: The People's Republic of China: Y. Shun-Zhang, Public Health School, Shanghai Medical University, Shanghai, and W. Blot, National Cancer Institute, Bethesda, MD; Thailand: P. Srivatanakul, National Cancer Institute, Bangkok; The Gambia: S. J. Allen and H. Whittle, MRC, Fajara, The Gambia; Kenya: D. Forman, ICRF, Oxford, UK; Senegal: P. Coursaget, Institute of Virology, Tours, France.

[b]Limit of detection, 5 pg AFB_1–lysine Eq/mg albumin.

and it is important to confirm such findings. The sensitivity of the aflatoxin-albumin assay should allow detection of the levels of aflatoxin exposure required to yield the liver DNA adduct levels reported, unless the latter accumulate over many years, and it would be valuable to assess the serum albumin adducts in these individuals or in the populations from which they are drawn. No evidence of free AFB_1 in a series of sera from the UK was found (*27*).

Second, seasonal variations in exposure may occur as seen in The Gambia in November and May (*17*). This requires further consideration in the design of future field studies. Third, striking geographical variations in exposure occur within a country In the Guangxi region in the Southeast of China, 70% of subjects had detectable levels of adduct, whereas no subjects from the northeastern province of Shandong were positive (unpublished collaborative studies with J. Chen, Institution of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing, and S. Z. Yu, Epidemiology Department, Public Health School, Shanghai Medical University, Shanghai).

Exposure to aflatoxin has been observed in all age groups that we have examined to date. Exposure was as high in Gambian children (age 3–8 years) as in adults (*17*), and it is also known that aflatoxin exposure at earlier ages can occur via breast milk (*28,29*). In the latter case, metabolism of aflatoxin by the mother reduces the child's exposure to less than 1% of the mothers dietary intake (*29*), and the metabolites found in the milk, although carcinogenic, are less so than AFB_1 (*30*). Recently it was also demonstrated that transplacental exposure to aflatoxin occurs in this population with levels of aflatoxin–albumin adduct in umbilical cord blood being 5–10 times lower than in the mothers' venous blood at the time of delivery (*31*). This *in utero* exposure to a mutagen, as well as exposure soon after birth,

could be particularly relevant because the putative stem cell population ("oval cells") could be mutated during the period of liver cell differentiation (*32*). Subsequently, the cell proliferation occurring in the liver of HBV carriers with chronic, active hepatitis could lead to the clonal expansion of these initiated cells and to the occurrence of other genetic changes leading to cancer. It is of interest that in populations where aflatoxin exposure is high, a repeated observation is the high prevalence of PHC compared to hepatoblastoma in teenage children (*33–35*), whereas the latter is normally more common in children.

The overall pattern of aflatoxin exposure in The Gambia, for example, is one of chronic exposure throughout the lifetime of the individual, at the same high level except for some reduction during the perinatal period. The percentage of the population who are chronic carriers of HBV is also at a maximum by 2–3 years of age in the Gambia (*36*). These data on aflatoxin exposure and HBV infection are summarized together with the age-dependent incidence of hepatocellular carcinoma in The Gambia in Figure 1. Exposure to aflatoxin can therefore precede HBV infection in The Gambia, and the two risk factors are present from an early age, giving the maximum time period within which they could interact. Data from animal model and field studies directly pertaining to the question of whether aflatoxin and and HBV do interact are presented below.

Animal Models

The interaction of aflatoxin and HBV has been examined in two of the animal models for which hepadna viruses have been identified, namely, the Pekin duck and woodchuck (*37*). In addition, various strains of transgenic mice expressing providing an different human HBV proteins have been developed (*38–40*), providing an alternative approach. A study of acute viral infection of marmosets (*Saguinus oedipomidas*) treated with AFB_1 has also been reported (*41*).

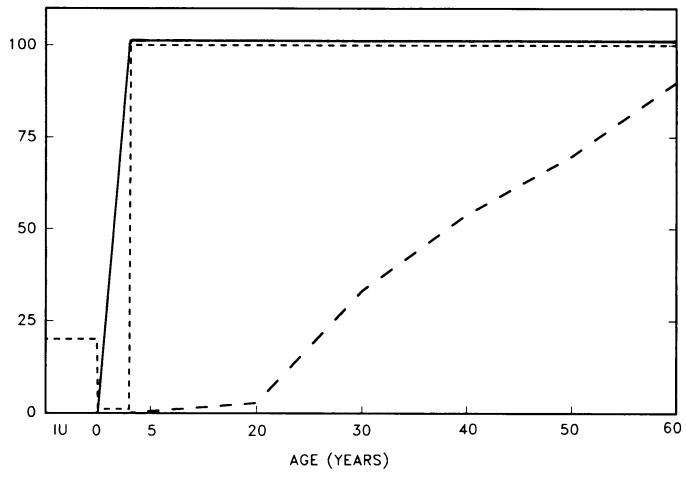

FIGURE 1. Schematic representation of aflatoxin exposure, hepatitis B virus (HBV) infection, and liver cancer incidence in The Gambia. The Y-axis represents the percentage of the rate for the adult population for aflatoxin exposure (-----) and HBV infection (—). The 100% adult rate in The Gambia for chronic Hepatitis B virus surface antigen carriers was taken as 14% (*36*), and the aflatoxin exposure data are from Wild et al. (*17,31*). The male age-specific liver cancer rates per 100,000 (---) for The Gambia are from Bah et al. (*72*), and in this case the Y-axis is this incidence rate per 100,000. IU, *in utero*.

Carcinogenicity Studies

Three studies have been published in which the carcinogenicity of AFB_1 has been compared in HBV-infected and noninfected ducks (summarized in Table 2). In our laboratory, two doses of AFB_1 were used with a weekly administration. While an increased mortality and more marked nontumor liver pathology were observed in HBV-infected birds given AFB_1 compared to those without the carcinogen treatment, no statistically significant differences in liver cancer induction occurred (*42*). Other studies (*43,44*) used different treatment regimens with higher doses of AFB_1 but also reported no significant difference in tumor formation in ducks administered AFB_1 in the presence or absence of HBV infection.

In all three studies the number of ducks is low, limiting the strength of the conclusion, but taken overall the two factors do not appear to act synergistically or additively to produce liver tumors in this model. This may reflect the relatively mild liver pathology observed in chronically infected ducks, with an absence of immune-mediated destruction of HBV-infected hepatocytes (*44*), compared to that seen in woodchucks (*45*) or humans. No liver tumors occurred in HBV-infected ducks in the absence of AFB_1 in the above experiments, and such cases have only rarely been reported elsewhere (*46,47*). In the latter cases, dietary exposure to aflatoxin could not be excluded. In contrast, in the woodchuck, liver tumors occur in almost 100% of HBV-infected animals (*48*). Carcinogenicity studies with AFB_1 and HBV in woodchucks would be informative.

In transgenic mice, two studies have described the interaction between chemical carcinogens and HBV in the induction of liver tumors, and these are summarized in Table 3. The most convincing evidence to date from animal models for a synergism between AFB_1 and HBV in the induction of liver tumors is the work of Sell et al. (*49*). These authors used transgenic mice containing an HBV DNA fragment coding for the entire HBV surface antigen (HBsAg) under transcriptional control of the mouse albumin gene promotor (*39*). In this line of transgenic mice there is an accumulation of HBV large-envelope protein in the hepatocytes, resulting in severe, chronic liver damage. All these mice develop PHC, in the absence of other treatment, at 20 months of age (*50*). When treated with AFB_1, a highly significant number of adenomas and PHC were observed at 15 months in the transgenic mice compared to an absence of such tumors in untreated transgenic mice (Table 3). Similar results were ob-

Table 2. Studies on the role of AFB_1 and HBV infection in induction of liver cancer in Pekin ducks.

AFB_1, mg/kg	HBV[a]	No. of animals with tumors	Reference
0.08	–	3/10 (3 HCC, 1 ADE)	Cova et al., 1990 (*41*)
0.08	+	3/6 (3 HCC)	
0.02	–	2/10 (1 HCC, 1 ADE)	
0.02	+	0/13	
0.2	–	3/4 (3 HCC)	Cullen et al., 1990 (*43*)
	+	6/8 (3 HCC, 1 BC, 3 ADE)	
0.1	–	2/8 (2 HCC)	Uchida et al., 1988 (*42*)
0.1	+	0/8	

Abbreviations: AFB_1, aflatoxin B_1; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ADE, adenocarcinoma; BC, biliary carcinoma.

[a]Ducks were either congenitally infected (*42,44*) or inoculated with HBV after hatching (*43*).

Table 3. Chemical hepatocarcinogenesis in HBV-transgenic mice.

Plasmid recombinant	Strain (sex)	Carcinogen treatment	No. of mice	No. mice with tumors ADE	PHC	Reference
HBV only	C3H/He (M)	DEN	23	9	3	Dragani et al., 1989 (*54*)
None		DEN	19	5	1	
HBV only	C3H/He (M)	DAB	12	5	1	
None		DAB	22	7	—	
HBV+Alb promoter	C57Bl/6 (F)	AFB_1[a]	8	10[b]	2	Sell et al., 1991 (*48*)
None		AFB_1	8	—	—	
HBV+Alb promoter	C57Bl/6 (F)	DEN[c]	8	9	2	
None		DEN	10	—	—	
HBV+Alb promoter	C57Bl/6 (F)	Phenobarbital[d]	4	—	—	
None		Phenobarbital	5	—	—	

Abbreviations: HBV, hepatitis B virus; Alb, albumin; DEN, dimethylnitrosamine; DAB, dimethylamino-azobenzene; AFB_1, aflatoxin B_1.

[a]Treatment was 2 μg/g AFB_1, administered three times at approximately 1-week intervals.

[b]Total number of PHC and adenomas in each group is recorded.

[c]Treatment was 50 μg/g DEN administered as a single dose.

[d]Treatment was 0.1% phenobarbital in the diet for 1 year.

tained with another hepatocarcinogen, diethylnitrosamine. The characteristic of the line of transgenic mice used in this study is the toxic concentration of HBsAg in the hepatocyte, which leads to direct damage to these cells. In humans, the liver cell damage due to HBV infection and the resultant cellular proliferation are probably due to mechanisms other than the accumulation of HBsAg seen in the above model. Nevertheless, a common observation is that liver damage resulting from a range of insults that induce cell proliferation, e.g., HBV, hepatitis C virus (HCV), alcohol, genetic disorders, is associated with development of PHC in humans (*51–54*), and therefore this model may be representative of the human situation.

Dragani et al. (*55*) reported a less marked but significant increase in liver tumors in a different line of transgenic male mice treated with diethylnitrosamine and another hepatocarcinogen, dimethylaminoazobenzene. In these mice no liver pathology results from the presence of the transgene (*38*), and the increased carcinogenicity is seen in the absence of liver damage. Another difference in this study compared to that of Sell et al. (*49*) was that a single dose of carcinogen was administered at 7 days of age, whereas in the latter study carcinogen treatment started at 3 months, a time at which moderately severe chronic hepatitis is already present (*50*).

One study in marmosets reports the interaction between AFB_1 (200 μg/kg body weight; 5 days per week) and viral hepatitis (*41*). Infection was acute using an inoculum from a human hepatitis case that had been serially passaged in marmosets (*56*), and animals were infected 6 weeks after the start of AFB_1 treatment. Of 12 marmosets given AFB_1 alone, one developed a PHC after 50 weeks, whereas in the group having the viral infection in addition, 2 of 12 developed PHC after 87 weeks. Cirrhosis and portal fibrosis were most severe in the group receiving AFB_1 and hepatitis infection. No tumors were seen in animals receiving only virus or in untreated animals. It was also observed that cirrhosis was induced in the marmosets with AFB_1, whereas in the rat this is not the case.

Carcinogen Metabolism

One hypothesis regarding a mode of interaction between aflatoxin and HBV is that viral infection could alter aflatoxin metabolism, either the activation to the reactive epoxide or the detoxification reactions (conjugation to glutathione, hydroxylation, etc.). Some early work with human liver biopsies suggested an increased *in vitro* activation of AFB_1 in cases of mild, chronic, active hepatitis but not in more severe cases (*57*). These studies have been more extensively pursued in woodchucks and the results recently summarized (*58*). A statistically significant increase in activation of the tryptophan pyrolysate, Trp-P-2, was observed in HBV carriers, and a higher but not significant activation of AFB_1 was reported. In addition, the level of reduced glutathione in livers of HBV-positive animals was lower, which could contribute to a higher exposure of DNA to active carcinogen metabolites.

In a preliminary experiment in Pekin ducks *in vivo*, we observed a lower level of AFB_1-DNA adducts in the livers of chronically HBV-infected compared to noninfected birds after a single dose of AFB_1 (*42*). In more recent studies we used newly hatched Pekin ducklings. Ducklings were divided into four groups of five ducklings with groups 1 and 3 infected with HBV on day 3 after hatching. On days 2 to 9 inclusive, groups 1 and 2 received 2 μg AFB_1/kg body weight, while groups 3 and 4 received solvent only. All ducklings were treated with [^{3}H]AFB_1 on day 13 (2 μg/kg) and were sacrificed 24 hr later. The amount of aflatoxin-adducts in serum albumin and liver DNA were quantitated by determining the level of radioactivity bound to these macromolecules, and the data are presented in Table 4. Viral infection had no effect on adduct levels in liver DNA. This is in contrast to the data in chronically infected ducks (*42*) mentioned above. There was a trend toward higher serum albumin binding in HBV-infected ducklings, but these levels are affected by a significant decrease

Table 4. Studies of the interaction of HBV infection and aflatoxin exposure in Pekin ducks.[a]

Group	Pretreatment AFB_1	Pretreatment HBV	Liver DNA adduct, pg AF/mg DNA ± SEM	AF-serum protein adduct, pg AF/mg protein ± SEM	Serum protein, mg/mL ± SEM	HBV-DNA, ng DHBV-DNA/mg liver DNA ± SEM
1	+	+	34.94 ± 5.6	15.1 ± 1.7	24.3 ± 1.9	20.1 ± 3.7
2	+	−	32.14 ± 4.4	11.9 ± 1.1	32.7 ± 1.8	
3	−	+	23.71 ± 3.7	15.2 ± 1.7	21.5 ± 1.2	24.0 ± 3.8
4	−	−	26.52 ± 3.1	14.0 ± 0.7	28.4 ± 1.4	

Abbreviations: HBV, hepatitis B virus; AFB_1, aflatoxin B_1, AF, aflatoxin.

[a]Treatment protocols are described in the text. AFB_1 was administered IP in dimethylsulfoxide (2 μg AFB_1/mL DMSO; 1 mL per kg body weight). Viral DNA was determined by denaturation and neutralization, followed by DNA-spot hybridization as described by Cova et al. (*42*). Statistical analysis was by analysis of variances (ANOVA test).

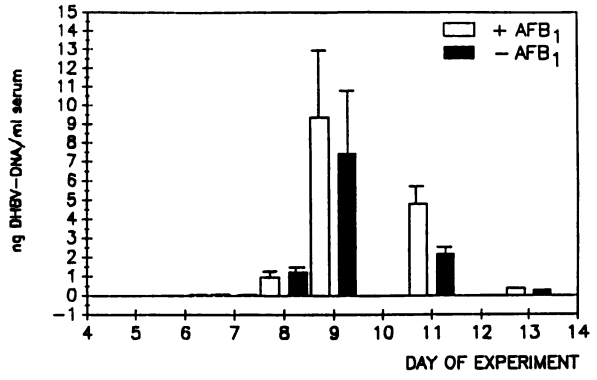

FIGURE 2. Variation of the mean serum duck hepatitis B virus–DNA concentrations with time. The experimental details are given in the text.

in serum protein concentrations in infected ducklings (Table 4). The pretreatment with AFB_1 increased the aflatoxin liver DNA binding, although the difference is only of borderline significance ($p = 0.06$; groups 1 and 2 compared to 3 and 4). This finding is different from observations in rats where pretreatment with AFB_1 led to a decrease in AFB_1-DNA adducts in the liver, in this case as a result of increased glutathione-*S*-transferase levels (*59,60*).

The above results are inconclusive as to the influence of HBV on the balance of aflatoxin activation and detoxification. As the cytochrome P-450 enzymes involved in aflatoxin metabolism are identified and noninvasive markers become available for use *in vivo* (*61,62*), studying the influence of HBV on aflatoxin metabolism is becoming more feasible.

Other Parameters

Little information is available on other potential ways in which aflatoxin and HBV may influence each others' biological effects. One hypothesis is that AFB_1 exposure could alter viral replication and/or integration as has been discussed for other viral/chemical interactions (*63*). In our study of ducklings described above, we also measured HBV-DNA in the sera and livers of ducklings that received AFB_1 from one day prior to HBV inoculation and for 6 days after inoculation. There were no differences in the daily mean levels of HBV-DNA in the serum (Fig. 2), in the maximum serum viral concentrations (seen on days 9-11 after hatching) or in the concentration at the time of sacrifice on day 14 (data not shown). The viral DNA per microgram of liver DNA was also determined on day 14 (Table 4) but no significant differences were found. Again these results were in contrast to our observations in chronically infected adult ducks where viral DNA titers in the sera and livers of AFB_1-treated ducks tended to be higher than untreated ducks (*42*). These results merit further investigation, taking into account the immune status of the ducks, the liver pathology, and the effects of chronic AFB_1 exposure. Of interest is the report by Ronai and Weinstein (*64*) that DNA damage induced by UV irradiation can induce a *trans*-acting protein that alters viral DNA replication.

Field Studies in Humans

There are indications of an interaction between aflatoxin and HBV from epidemiological studies. Several studies have shown geographical correlations between dietary AFB_1 and PHC in areas where the HBsAg carrier rates are not significantly different (*5,6*). In addition, an important recent study showed a synergistic interaction between HBV and aflatoxin in a prospective cohort study in China (*65*). Another interesting study showed an earlier onset of PHC in rural compared to urban South African Blacks with similar HBV status in the two groups (*35*). All subjects had spent the early part of their lives in rural areas but the "urban" group had migrated to the cities in late adolescence or early adulthood. The suggestion was made that greater likelihood of exposure to a co-carcinogen such as aflatoxin could occur in a rural setting. This suggests that in addition to the importance of aflatoxin acting at an early stage of hepatocarcinogenesis, it may also act at a later stage. The observations of earlier tumor onset in HBV carriers exposed to AFB_1 (or another carcinogen) are of interest with respect to the data of Sell et al. (*49*) mentioned above. Other studies in Mozambique have also suggested an action of aflatoxin at a late stage of hepatocarcinogenesis (*34*). The *p53* mutation spectra (*66*) in liver tumors occurring in different age groups could be informative in assessing the role of aflatoxin and HBV infection in the various stages of PHC development.

Our laboratory has investigated HBV and aflatoxin exposure in two studies in The Gambia, West Africa. In a study of 323 children in the Farafenni region of the country, we examined the association at the individual level between aflatoxin–albumin adducts, cell-mediated immune response to malaria antigens, HBV status, malaria infection, and ethnic group (*67*). Twenty-five of 323 children (age 3–8 years) were positive for HBsAg, and the aflatoxin–adduct levels were higher in carriers (log mean = 4.41, SD = 0.95 pg AFB_1lysine Eq/mg albumin) than noncarriers (log mean = 4.01, SD = 1.05; *t* -test: $t = 1.78$, $p = 0.08$). Multiple regression analysis was performed to allow for confounding variables, and the effect of HBsAg was then at the limit of statistical significance ($p = 0.05$). This observation suggests that current HBV infection increases the activation of aflatoxin, formation of aflatoxin–albumin adducts, and consequently the level of DNA damage in the liver. These results are of interest with respect to the studies performed in woodchucks on the influence of HBV on AFB_1 metabolism.

In a second study (*68,69*) in The Gambia, a detailed analysis of dietary intake of aflatoxin over a 7-day period was compared with the level of albumin-bound aflatoxin and the urinary excretion of aflatoxin-7-guanine adduct in a group of 20 individuals. Of the study subjects, 9 were chronic HBsAg carriers and 11 were noncarriers, and where possible the subjects were paired for age, sex, and food intake to examine the hypothesis as to whether, for a given intake of aflatoxin, the level of aflatoxin–protein and/or DNA adduct formation was different in these two groups. In fact, in both the level of aflatoxin–albumin adduct (*69*) and the excreted aflatoxin-7-guanine adduct (*68*) there was no difference between carriers and noncarriers as groups when adjusted for dietary exposure, although there was considerable interindividual variation. The small numbers of individuals in this study should be noted. The study of Allen et al. (*67*) also demonstrated a highly significant difference in aflatoxin–albumin adduct levels in three major ethnic groups of The Gambia, which could have a genetic basis in differences in AFB_1 metabolism.

The possibility that AFB_1 could act via an immune suppression resulting in an increased susceptibility to chronic HBV in-

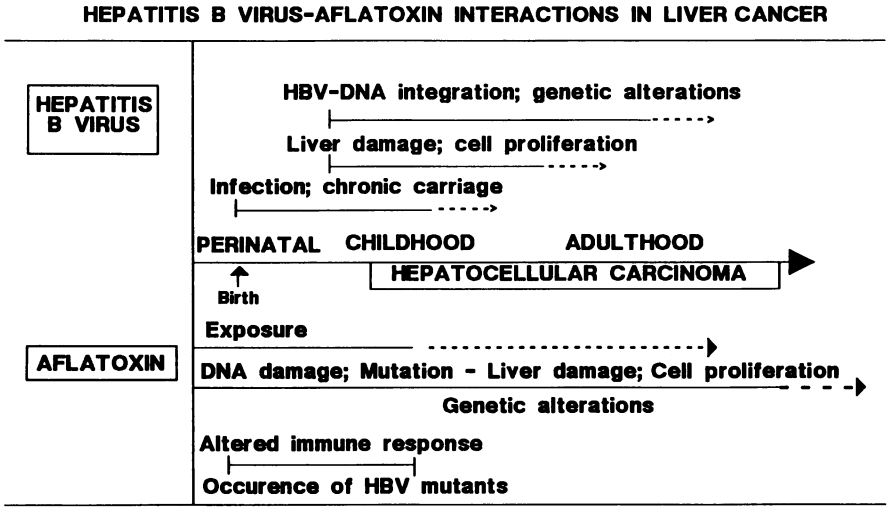

FIGURE 3. Aflatoxin, hepatitis B virus, and primary hepatocellular carcinoma: possible interactions.

fection has been a long-standing hypothesis (*70*). However, despite considerable data on the immune-suppressive action of AFB_1 in animals and subsequent viral or parasitic infections (*71*), to our knowledge no studies have specifically addressed this question in animals with hepadna virus infection.

In the study by Allen et al. (*67*), the level of aflatoxin–albumin was not related to any consistent effect on *in vivo* lymphoproliferative responses. *In vivo* malaria-specific antibody titers tended to be inversely correlated with aflatoxin–adduct level, but this was not statistically significant. Thus, these data did not provide any evidence for an effect of aflatoxin exposure on the immune response in these children. However, the marker that we used for aflatoxin exposure is relatively short term (2–3 months), and this exposure period may not have been relevant to the immune status measured at the same time. Far more extensive work is required to resolve this question, especially given the positive observations in animals (*71*).

Conclusions

The interaction between AFB_1 and HBV is an attractive proposal to explain the epidemiological observations on PHC in high risk areas of the world, and several potential mechanisms have been proposed. The data for these are summarized above and represented schematically in Figure 3. The actions of both agents may be mutagenic and mitogenic at various stages of the carcinogenic process with the additional possibility of immune suppression by aflatoxin. Given the various effects of these two agents on a cell, no one specific mechanism should be expected to explain all cases of PHC. Despite the plausability of an interaction between HBV and aflatoxin, from epidemiology and the studies in transgenic mice, no strong evidence of a mechanism of interaction has been produced. However, with the availability of experimental systems and molecular approaches applicable to field studies, the tools to address this important question are now available.

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