Future MICROBIOLOGY

EDITORIAL

Aflatoxin: does it contribute to an increase in HIV viral load?

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"...mycotoxins, the most potent of which being the aflatoxins, must be considered to feature prominently in HIV disease progression."

HIV infects cells of the immune system, resulting in an initial major drop in CD^+ T cells and eventual depletion of these cells after a varying number of years, unless there is intervention with antiretroviral therapy (ART). A concomitant increase in HIV viral load and progression to AIDS occurs with depletion of $CD4^+$ T cells. Our research among HIV-positive people in Ghana, West Africa, consistently shows an increase in HIV viral load in those with higher aflatoxin B_1 (AFB_1)-lysine adduct levels in their blood [1–3]. Although our studies have been ambidirectional or prospective in design, so far we have assessed aflatoxin and viral load levels at recruitment [2], or have reported baseline aflatoxin and viral load levels [3]. We were careful in our latter study to recruit antiretroviral-naive HIV-positive people with high median and mean CD4 counts (median: 574 cells/µl of blood; mean ± standard deviation: 630 ± 277), so that the effect of HIV/AIDS-related clinical conditions or opportunistic infections, and the use of ART, could be eliminated as possible factors contributing to the observed association between high aflatoxin levels and viral loads [3]. Therefore, we have to err on the side of caution and posit that aflatoxin contributes to increases in HIV viral load. Our results also indicate that the effects of aflatoxin on viral load occur early in HIV infection, even well before $CD4^+$ T cells drop below 500 cells/µl of blood.

Aflatoxin could be increasing HIV viral load by its action in immune suppression, by increasing the rate of HIV proviral DNA transcription, or by both of these mechanisms, as well as by some other mechanism(s). Although the molecular mechanism(s) of immunomodulation by aflatoxin and other mycotoxins has not yet been completely delineated, available evidence suggests that immunosuppression by several mycotoxins occurs from inhibition of DNA, RNA and protein synthesis [4]. The AFB_1-8,9-epoxide, which is formed from AFB_1 by CYP450 oxidation, is highly reactive and can bind to DNA, RNA and proteins, forming adducts. Any alteration in nucleic acid structure caused by these adducts will impair DNA and RNA template activity, resulting in inhibition of the nucleotides and, ultimately, protein synthesis. Furthermore, AFB_1-epoxide binding to proteins may affect structural as well as enzymatic protein functions. The cells

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"If aflatoxin is contributing to increases in HIV viral load, the full impact of antiretroviral therapy is not being and will not be attained."

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of the immune system are continually proliferating and differentiating, and so are vulnerable to the immunomodulatory, primarily immunosuppressive, effects of the AFB_1-epoxide. We have observed decreases in certain immune cell subsets (significantly lower percentages of $CD4^+$ Tregs, perforin-expressing $CD8^+$ T cells and B cells) in those with high AFB_1-lysine adduct (AF-ALB) levels compared with those with low AF-ALB [1]. Impairments in these immune parameters could result in less effective control of HIV replication, resulting in increased viral load. We found that the HIV-positive group with high AF-ALB had the lowest percentage of Tregs of all groups, suggesting that there is a loss of Tregs in HIV-infected people with high AF-ALB. Tregs play a critical role in limiting immunopathology from persistent high-level immune stimulation in chronic viral infections [5]. Thus, the loss of Tregs may facilitate the immune hyperactivation associated with HIV and a high rate of viral replication. The lower percentage of perforin-expressing $CD8^+$ T cells found in HIV-infected people with high AF-ALB compared with those with low AF-ALB [1] indicates that the function of $CD8^+$ T cells synthesizing perforin in killing HIV-infected cells is impaired in people with high AF-ALB. This could lead to higher HIV viral loads.

Many animal studies show that aflatoxin exerts its immunomodulatory effects by modulating cytokine production by monocytes and macrophages [6]. A more recent study reported upregulation of expression of four proinflammatory cytokines (TNF-α, IL-1β, IL-6 and IFN-γ) and a regulatory cytokine (IL-10) in the spleen of pigs exposed to aflatoxin in feed. IL-6 was shown to impair antigenic- but not mitogenic-induced proliferation of lymphocytes to vaccination with ovalbumin, indicating that AFB_1 exposure decreases cell-mediated immunity, while inducing an inflammatory response [7]. Other researchers have shown that IL-6 prevents dendritic cell maturation [8] and that IL-10 inhibits the antigen-presenting function of macrophages and dendritic cells [9]. Thus, by keeping dendritic cells in an immature state, these cytokines could impair T-cell activation and the immune response. Several other studies conducted in animal and human monocytes/macrophages have shown that AFB_1 suppressed macrophage phagocytic activity (reviewed in [10]).

The second mechanism by which AFB_1 may increase HIV viral load is through increasing proviral DNA transcription. Yao *et al.* reported that AFB_1 and two other toxicants, benzo[a]pyrene (BaP) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), significantly increased the chloramphenicol acetyltransferase reporter gene linked to the promoter sequences in the HIV-1 long terminal repeat, thereby increasing the rate of proviral transcription [11]. Pokrovsky *et al.* [12] and Tsyrlov and Pokrovsky [13] had previously shown that TCDD increased infectious HIV-1 titers in human lymphoid cell culture. However, Yao *et al.* found that AFB_1 and BaP were more potent than TCDD in increasing CAT activity [11]. Although the mechanism by which AFB_1 increased HIV-1 transcription was not investigated, Yao *et al.* showed that induction of a functional CYP1A1 monooxygenase by TCDD was necessary to stimulate thiol-sensitive reactive oxygen intermediates responsible for the TCDD-dependent activation of genes linked to the HIV-1 long terminal repeat [11]. AFB_1 is not a polycyclic aromatic hydrocarbon like TCDD and BaP, but is similar to BaP in carcinogenic potency and ability to form guanine adducts in DNA. Thus, AFB_1 and BaP may function similarly to TCDD in increasing HIV-1 transcription and hence HIV viral load.

Aflatoxin may also indirectly contribute to HIV replication through its effect in decreasing micronutrient levels, as well as protein synthesis and food conversion efficiency. Sub-Saharan Africans are disproportionately burdened by malnutrition and deficiencies of nutrients (such as vitamins A, B, C and E), which have been implicated in HIV transmission and progression, and death [14,15]. Micronutrient malnutrition further impairs the immune system by suppressing immune function necessary for survival. We found significant inverse associations between vitamin A and E levels, and HIV viral load [16]. This suggests that aflatoxin exposure significantly compromises the micronutrient status of people who are already facing overwhelming health problems associated with HIV infection. Vitamin A is a fat-soluble micronutrient that is essential for immunity, cellular differentiation, maintaining epithelial surfaces, growth, reproduction and vision. Vitamin E is also important in immune function, and low serum vitamin E was found to be associated with HIV disease progression in prospective studies [15]. Vitamin E is an antioxidant that reduces oxidative stress. High levels of oxidants in lymphocytes could lead to viral activation and an increase in HIV viral load.

Aflatoxin may contribute to increases in HIV viral load through its significant role in liver injury. Aflatoxin targets the liver and induces injury to both the hepatic parenchyma and biliary tract. In addition to the important function performed by the liver in metabolizing toxins, alcohol and drugs, the liver stores many essential nutrients, vitamins (e.g., A, D, E, K and B12) and minerals (e.g., iron and copper), some of which are also important in immune function. In addition, Kupffer cells (a type of fixed macrophage that lines the liver sinusoids) form an important part of the immune system in phagocytizing and digesting microorganisms and cell debris. Therefore, it is conceivable that by impairing liver function, aflatoxin could contribute to increases in HIV viral load. Liver injury in HIV-positive people may also result from hepatitis B or C infections, alcohol use, certain antiretroviral drugs [17] and other medications, such as antituberculosis drugs. Such liver injury would further compromise the ability of the hepatocytes to inactivate aflatoxin, leading to an increase in levels of the toxin and its harmful health effects.

Implications of an increase in HIV viral load by aflatoxin

Aflatoxin contamination of staple crops affects approximately 4.5 billion people living in developing countries of Africa, Asia and South America [18]. Coincidentally, progression to AIDS and death also occurs more rapidly among HIV-infected people in these high aflatoxin-exposure countries than HIV-positive people living in the USA, Europe and Australia, where aflatoxin levels are strictly regulated [19]. Although several factors, such as poor nutritional status, higher levels of infectious disease episodes, lack of prophylactic treatment for opportunistic infections and exposure to other environmental toxins, could be contributing to the more rapid rate of HIV progression and AIDS in developing countries, mycotoxins, the most potent of which being the aflatoxins, must be considered to feature prominently in HIV disease progression. The median time from HIV-1 seroconversion to clinical AIDS was 7.4 years in a group of Thai soldiers compared with 11 years for HIV-positive individuals from Europe, North America and Australia [19]. The mean time from HIV seroconversion to clinical AIDS in a study conducted in West Africa ranged from 5 to 7.2 years depending on the HIV-1 subtype [20].

ART has been rolled out effectively in most developing countries with high aflatoxin exposure levels. However, the number of people who need ART is staggering and difficult to meet. In 2011, only approximately 56% of HIV-positive people who needed ART in developing countries received it. In addition, new HIV infections occur daily (2.5 million in 2011, 1.8 million of which occurred in sub-Saharan Africa), thereby increasing the pool of people who will continue to need ART. Furthermore, even in the era of ART, 1.7 million people died of AIDS in low- and middle-income countries in 2011 [21]. If aflatoxin is contributing to increases in HIV viral load, the full impact of ART is not being and will not be attained. In fact, ART may be of little benefit to those most affected by exposure to high levels of aflatoxin. Since high HIV viral load translates into greater risk of HIV transmission, if WHO's targets of reducing new HIV infections by 50% and reducing HIV-related deaths by 25% by 2015 [22] are to be achieved, region- or country-specific problems associated with HIV infection and progression must be addressed. Aflatoxin exposure in HIV infected people is one such major problem!

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