

The impact of aflatoxin exposure on infectious disease: immune function, malaria and HIV-AIDS

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Aflatoxin and child health

Acute liver toxicity

Liver carcinogenicity

Growth impairment

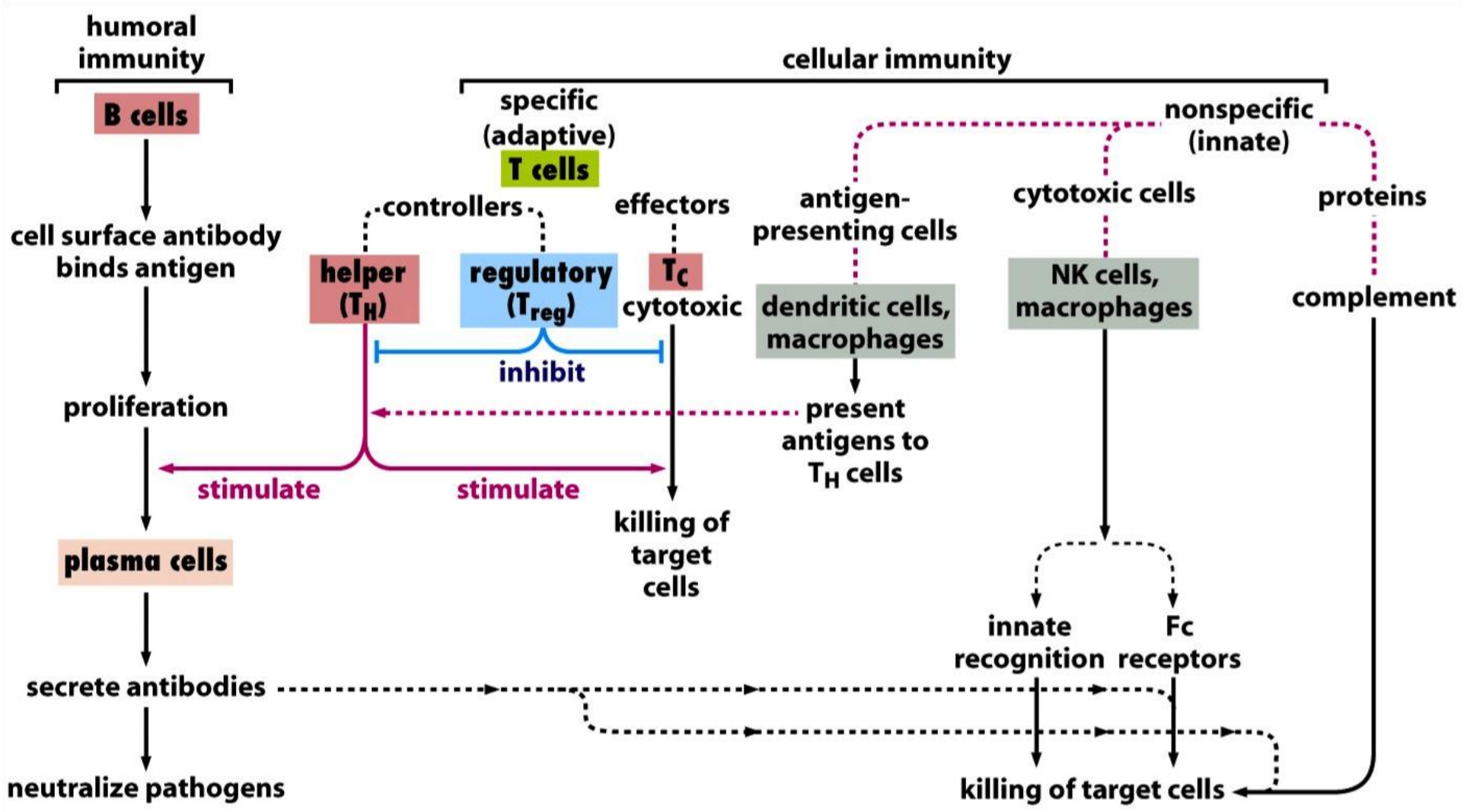


Hepatosplenomegaly

Immune suppression?

Increased susceptibility to disease?

Environmental enteropathy?



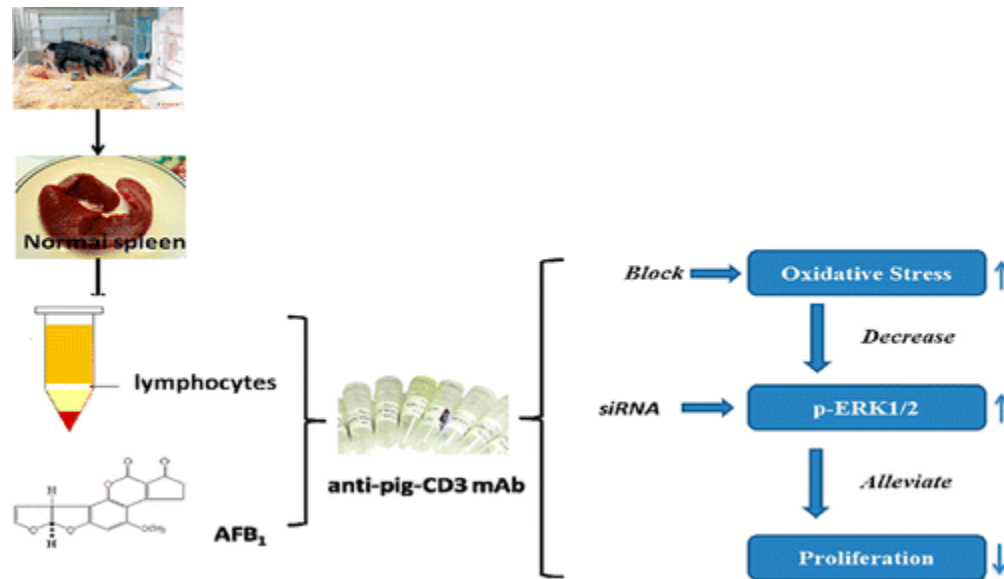
Aflatoxin impairs immune function in animals

Effect	Demonstrated in:	
Decreased B & T cell activity	Mice	Reddy et al, 1987
Impaired macrophage & neutrophil function	Poultry, mice, pigs	Neldon-Ortiz and Qureshi, 1992; Cusumano et al, 1996;
Modified synthesis of inflammatory cytokines	Mice, rats, pigs	Jakab et al, 1994; Meissonnier et al, 2008; Qian et al, 2014
Suppressed NK cell-mediated cytotoxicity	Mice	Reddy & Sharma, 1989
Decreased resistance to infectious disease	Poultry, pigs	Hamilton & Harris, 1971; Edds et al, 1973; Wyatt et al, 1975; Joens et al, 1981
Induced reactivation of chronic infection	Mice, poultry	Venurini et al, 1996; Kubena et al, 2001
Decreased immunity following vaccination	Poultry, rabbits	Gabal & Azzam, 1998; Gabal & Dimitri, 1998

Results from in vitro studies	
NK cell cytolysis impaired	Liu et al, 2002
Macrophage phagocytosis and production of reactive oxygen species impaired	Liu et al, 2002
Reduced viability of haematopoietic progenitor cells	Roda et al, 2010
Anti-inflammatory IL-10 secretion reduced, pro-inflammatory IL-6 secretion increased	Bruneau et al, 2012
Antigen presentation function of porcine dendritic cells was disrupted by low level aflatoxin treatment	Mehrzhad et al, 2014
T cell proliferation inducing capacity of dendritic cells increased	Mehrzhad et al, 2015

T cell activation and proliferation are critical for cellular immune function

Mechanism of immunotoxicity of aflatoxin in primary porcine splenocytes shown to involve oxidative stress and ERK1/2 MARK signalling pathway



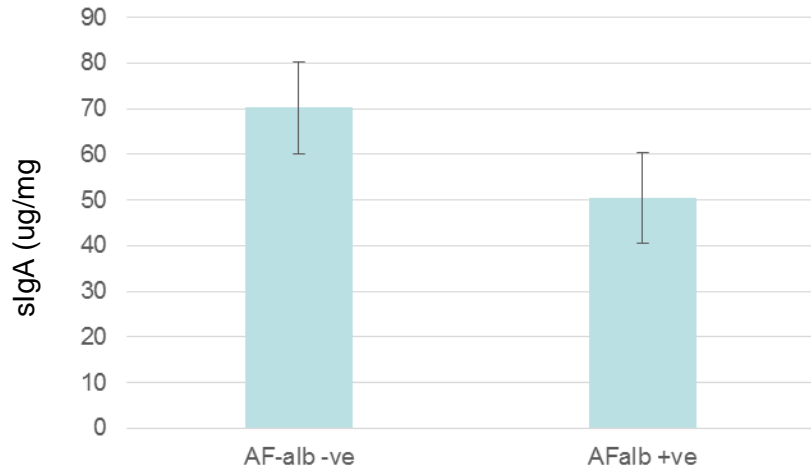
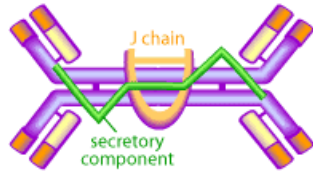
Hao et al (2015) J. Agri Food Chem **63**, 6094

Aflatoxin inhibited proliferation and IL-2 secretion in activated T cells
Inhibition of ERK1/2 or blocking oxidative stress countered these effects

Human studies

Ref	Study design/ Population Characteristics	Mycotoxin biomarker	Immune parameter/infectious disease marker	Results
Allen et al. 1992	<ul style="list-style-type: none"> - Gambia - Cohort - Sample size (n= 323) - Children 3-8 years 	Serum aflatoxin albumin adduct	<ul style="list-style-type: none"> • Malaria – <i>P. falciparum</i> • Antibody titre – asexual stages of <i>P. falciparum</i> • HBV – HBSAg 	<ul style="list-style-type: none"> • Mean AFB1 was higher in children with <i>P. Falciparum</i> parasitaemia (n=102) than children with no parasitaemia (n= 221) P = 0.06. • No significant associations with experience of malaria infection, antibody titre to asexual stages of <i>Plasmodium falciparum</i> or lymphoproliferative responses • AFB1 levels were higher in children who were HbsAg +ve than those who were HbsAg -ve
Turner et al. 2003	<ul style="list-style-type: none"> - Gambia - Cohort - Sample size (n=472) - Children 6-9 years (251 male, 221 female) 	Serum aflatoxin- albumin adduct	<ul style="list-style-type: none"> • Secretory IgA in saliva (sIgA) • Cell-mediated immunity (CMI) • Antibody responses to both rabies and pneumococcal polysaccharide vaccines 	<ul style="list-style-type: none"> • AF-alb levels detected in 93% of sample • Children with detectable levels of AF-alb had significantly lower sIgA compared to those with non-detectable AF-alb levels (P <0.0001) • There was no association between CMI responses to test antigens and AF-alb. • Only 1 of the pneumococcal antibody titers was associated with Af-alb (P = 0.05) the rabies antibody titre was not significant.
Jiang et al. 2005	<ul style="list-style-type: none"> - Ghana - Cross-sectional - Sample size (n = 64) - Adults 19 to 86 years - (34 males, 30 females) 	Plasma aflatoxin- albumin adduct	<ul style="list-style-type: none"> • Leukocyte immunophenotypes • T cells (CD3+) • subsets of T cells (CD4+ and CD8+) • B cells (CD19+) • NK cells (CD3-CD56+) • macrophages (CD14+) 	<ul style="list-style-type: none"> • AF-alb levels detected in 98% of sample • Participants with high AFB1 levels had significantly lower percentages of CD3+ and CD19+ cells (P = 0.002) and lower percentages of CD8+ T cells (P = 0.012) than participants with low AFB1 levels • Correlation analysis showed that low levels of CD3+CD69+ and CD19+CD69 cells were significantly associated with high AFB1 levels • Similar results observed in a multivariate analysis

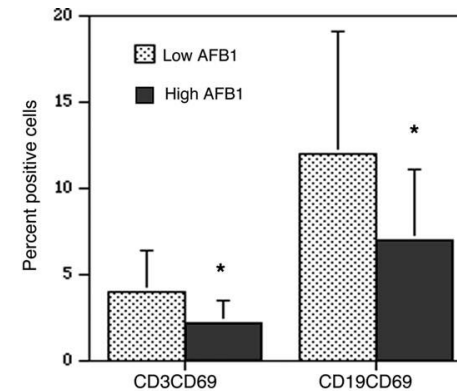
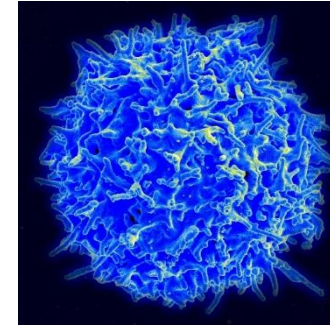
Turner et al, 2003
 High AF-alb } Lower sIgA in children



Test for cell mediated immunity response to antigen challenge showed no difference between high and low AF-alb

Only 1 out of 5 vaccination antibody titre response showed marginal significant difference with AF-alb

Jiang et al 2005
 High AF-alb } lower levels activated B and T cells



Also CD8 T cells that express perforin or granzyme were lower in patients with high AF-alb

Could lead to lowered immune response to infectious agents

Malaria

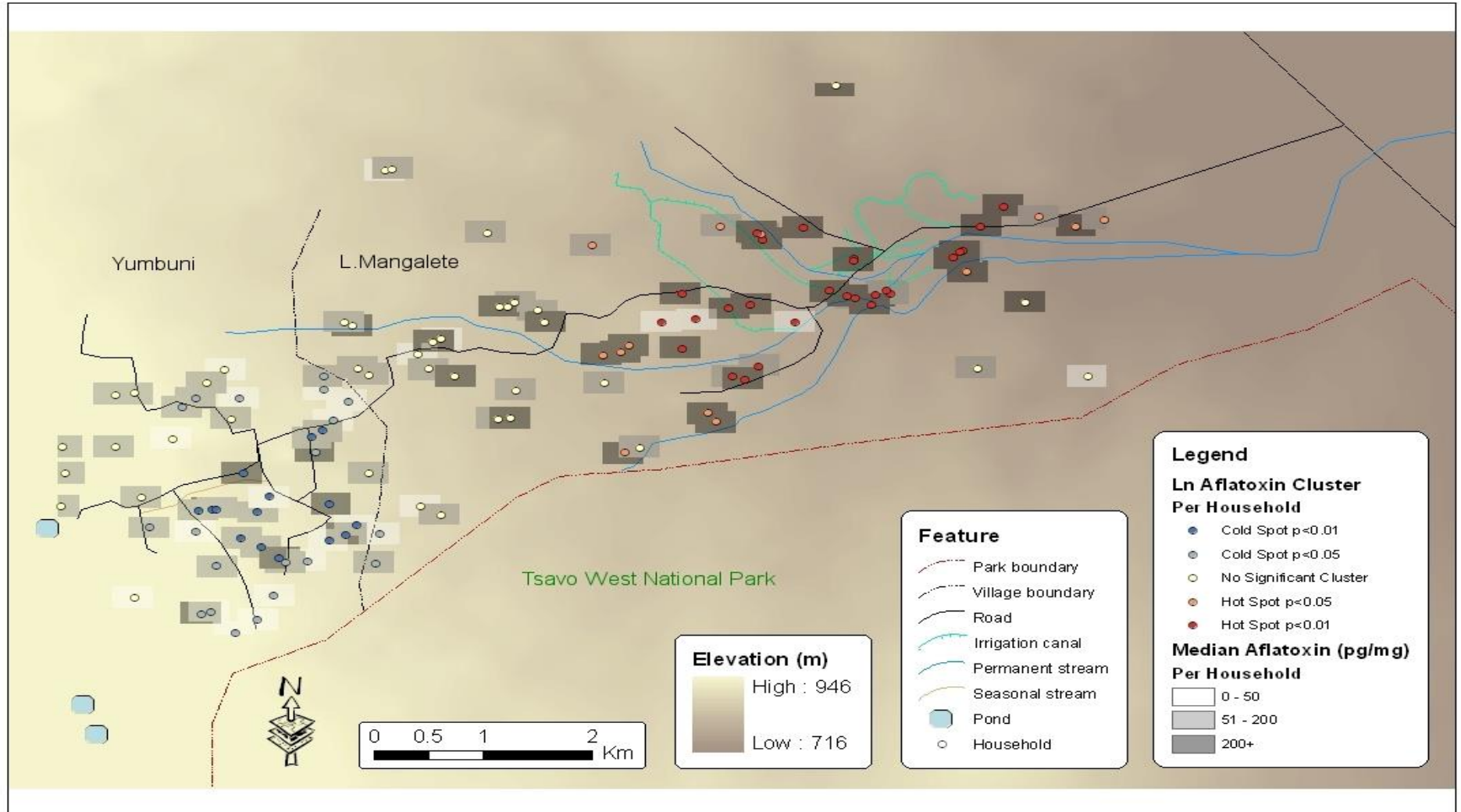


A study in Gambia in 1992 found an association between AF-alb and *Plasmodium* parasitaemia (Allen et al, 1992)

The association between AF-alb and anaemia in pregnant women in Ghana was stronger in women with malaria (Shuaib et al, 2010)

....but no association was found between AF-alb and malaria in Kenya in 2002 (Gong et al, 2012) or Ghana in 2007 (Jolly et al, 2007)

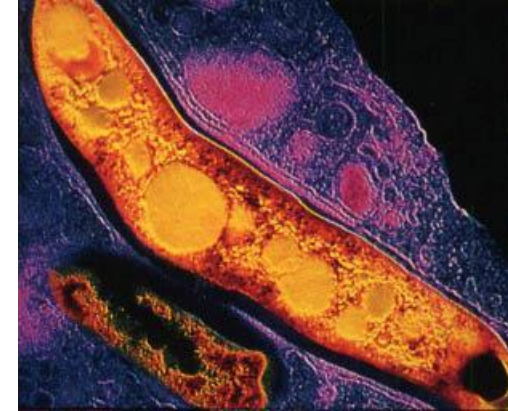
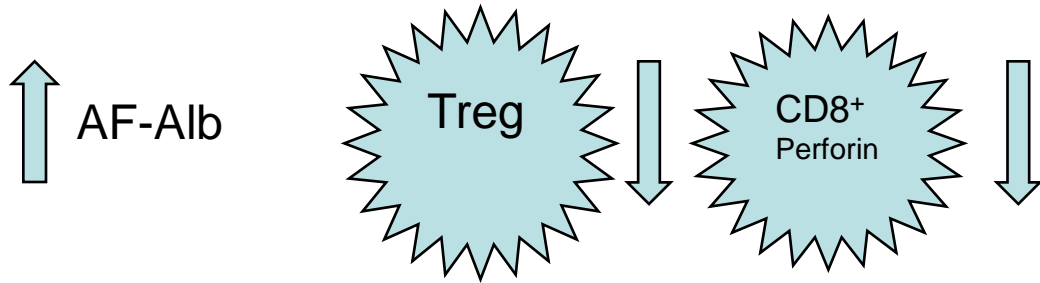
In Makueni, Kenya, high AF-alb was associated with hepatosplenomegaly



HIV

- HIV/AIDS is a huge problem in sub-Saharan Africa
- Aflatoxin induced immune suppression may contribute to HIV/AIDS in three ways:
 - Increased infection
 - More rapid progression of HIV
 - Reduced survivability with AIDS

Jiang et al, 2008
HIV+ve



Jiang et al, 2011
HIV+ve



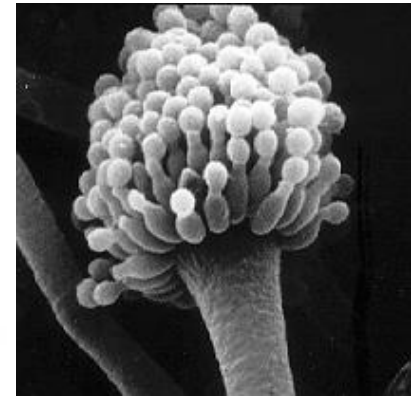
Keenan et al, 2011

Association between AF-alb
and TB infection in HIV+ve
patients

AF-alb associated with HIV+ve status

Reference	Study design/ Population Characteristics	Mycotoxin	Immune parameter/infectious disease marker	Result
Jiang et al. 2008	<ul style="list-style-type: none"> - Ghana - Cross sectional - Sample size (n= 196) - HIV positive: 38.25 ± 9.44 years - HIV negative: 40.77 ± 17.52 years 	Plasma aflatoxin-albumin adduct	<ul style="list-style-type: none"> • T cells (CD3+) • subsets of T cells (CD4+ and CD8+) • T cells phenotypes • B cells (CD19+) • NK cells (CD3-CD56+) 	<ul style="list-style-type: none"> • high AFB1 was associated with lower perforin expression on CD8+ T-cells (P = 0.012) • HIV+ve participants with high AF-ALB had significantly lower percentages of CD4+ T regulatory cells (P = 0.009) and naïve CD4+ T cells (p = 0.029) and reduced percentage of B-cells (P = 0.03) compared to HIV positive with low AF-ALB.
Jolly et al. 2011	<ul style="list-style-type: none"> - Ghana - Cross-sectional - Sample size (n= 314) - Adults (155 HIV-positive and 159 HIV-negative) 	Aflatoxin albumin adduct	<ul style="list-style-type: none"> - Viral load - CD4 count - liver function parameters - HBV, HCV and malaria infection parameters 	<ul style="list-style-type: none"> • Mean AF-ALB level was higher in the HIV+ve group compared with the HIV+ve group (P = 0.01). • HIV+ve participants with high AF-ALB levels(> 0.93 pmol/mg⁻¹ albumin based on group median) showed statistically significant increased odds of having higher HIV viral loads (OR = 2.84; 95% CI = 1.17 – 7.78) and higher direct bilirubin levels (OR = 5.47; 95% CI = 1.03 – 22.85) compared to the HIV+ group with lower AF-alb levels(<0.93 pmol/mg⁻¹). • CD4+ T cell counts did not differ significantly between the high AF-alb and low AF-alb group. • There were non-significant increased odds of being positive for HBV and malaria infection with higher AF-ALB levels.
Keenan et al. 2011	<ul style="list-style-type: none"> - Ghana - Longitudinal Cohort - Sample Size (n= 141) - HIV positive adults 	Aflatoxin albumin adduct	<ul style="list-style-type: none"> • CD4+ T- cells • Viral load • Malaria • tuberculosis • HBV • Pneumonia 	<ul style="list-style-type: none"> • Tuberculosis was the only disease with a significant finding with those in the highest AF-ALB quartile having a HR of 3.39 (95% CI 1.15 – 9.98, P = 0.03)

Epigenetic consequences of early life exposure to Aflatoxin



No differences in gene expression of immune system genes observed in children at 6 months associated with maternal aflatoxin exposure during pregnancy

Some aflatoxin related changes in DNA methylation in immune genes

CCL28 Chemokine (C-C motif) ligand 28

TLR2 Toll-like receptor 2

TGF- β 1 Transforming growth factor- β 1

Hernandez-Vargas H et al (2015)

Aflatoxin exposure and associated immune function damage in young children

ENID trial in Gambia

Nutrient supplementation in pregnancy

Thymus size at 6 & 12 months

Antibody response to vaccination (Diphtheria, tetanus toxoid, Hib Ab) at 6 & 12 months

Post-natal maturation and homeostasis of CD4 T cells and B cells (Prof David Lewis, Stanford University) at 12 months

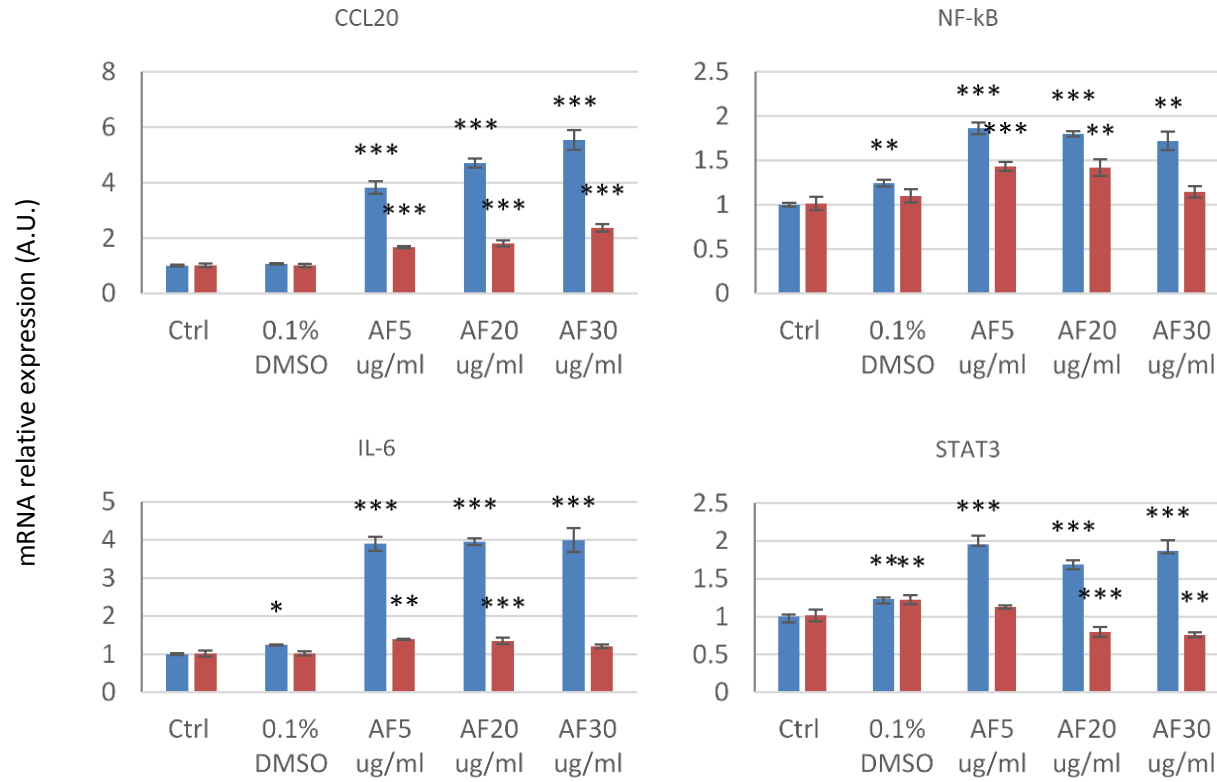
MORDOR-HG Trial, Malawi

Single dose azithromycin at 6 months, 3 month follow up

Intestinal inflammation (fecal neopterin)

Intestinal permeability (dual sugar test)

Immune activation (antibacterial response PCR)



Increased expression of immune related genes in AFB₁ treated HHL-16 cells

Immune suppression due to aflatoxin

- Aflatoxin causes disruption of the immune response and increases susceptibility to infection in several animal species
- Evidence from *in vitro* models reveals some of the mechanisms by which this immunotoxicity occurs
- There are currently only a few studies in humans showing an association between aflatoxin exposure and immune suppression
- High AF-alb may facilitate HIV associated immune hyperactivation and lead to more severe disease
- There is a need for further investigations:
 - Varied measures of immune response
 - Larger sample sizes
 - Longitudinal cohort studies
 - Repeat in different populations
 - Other mycotoxins (co-exposure)

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