

Effects of aflatoxins and fumonisins on the immune system and gut function

There are few studies in humans that provide information on the impact of aflatoxin on the immune system. Those available have provided suggestive evidence of effects of aflatoxin similar to those observed in relevant animal models (IARC, 2002; Turner et al., 2003; Wild and Gong, 2010). No studies are available of the impact on immune function in populations of children highly exposed to fumonisin or of co-exposure to aflatoxin. Given the prevalence of mycotoxin exposures in populations vulnerable to infectious diseases, there is a need for well-designed studies of the impact of aflatoxin and fumonisin, alone or in combination, on the immune system and intestinal integrity.

Aflatoxins

In vivo studies in pigs exposed to aflatoxin B₁ (AFB₁) suggest that cytokine

upregulation occurs at relatively low AFB₁ exposures (~0.9 mg/kg of feed) (Meissonnier et al., 2008). Interleukin-1 (IL-1) levels increased 1 day after dosing, due to production by peritoneal macrophages, in Fisher 344 rats given a single intraperitoneal injection of 1 mg/kg body weight (bw) AFB₁ (Cukrová et al., 1992). Also in Fisher rats, weaned animals were fed diets containing from 0 to 1.6 ppm AFB₁, 4 weeks on and 4 weeks off for 40 weeks, or the 1.6 ppm AFB₁ diet continuously (~0.1 mg/kg bw/day). The percentages of T and B cells in spleen were affected after the dosing cycles. Significantly increased production of IL-1 and IL-6 by lymphocytes in culture was seen in the second dosing cycle (12 weeks) and the second “off” cycle (16 weeks) at the higher doses. Inflammatory infiltrates were observed in the liver after 8 weeks

of continuous and intermittent dosing and were increased in size and number at 12 weeks in both 1.6 ppm dose groups. This correlated with peak production of IL-1 and IL-6 (Hinton et al., 2003).

Exposure of Fisher rats to AFB₁ at 5–75 mg/kg bw by gavage for 1 week showed dose-dependent decreases in the percentage of splenic CD8+ T cells and CD3–CD8a+ natural killer (NK) cells. A general inhibition of the expression of IL-4 and interferon-gamma (IFN-γ) by CD4+ T cells, of IL-4 and IFN-γ expression by CD8a+ cells, and of tumour necrosis factor alpha (TNF-α) expression by NK cells was also found. These data suggest that AFB₁ elicits inflammatory responses by inducing cytokine expression (Qian et al., 2014).

Studies in cell lines suggest that AFB₁ inhibits the viability of hematopoietic progenitors and IL-8-induced

neutrophil chemotaxis (Roda et al., 2010; Bruneau et al., 2012). These effects, although identified in vitro, are likely part of the mechanism for AFB₁-related impairment of phagocytosis and bactericidal activity observed in animal models in vivo. Altered white blood cell function is likely to result in a longer and more severe bacterial/fungal infection with greater inflammation. Elevated levels of pro-inflammatory cytokines have been reported in humans, associated with AFB₁ exposure (Jiang et al., 2005). However, it is not clear whether this upregulation is predominantly direct or indirect (as a consequence of prolonged infection/inflammation). Direct upregulation of cytokines might occur through increased transcription factor binding or increased cytokine messenger RNA (mRNA) stability. Another potential mechanism of cytokine upregulation is related to infection in a compromised host. An impaired immune system, in the context of AFB₁ exposure, has been associated with increased viraemia, parasitaemia, increased susceptibility to infection, and reduced response to vaccines in animals (Bondy and Pestka, 2000; Meissonnier et al., 2006).

The intestine functions as a selectively permeable barrier, placing the mucosal epithelium at the centre of interactions between the mucosa and luminal contents, which include dietary antigens, microbial products, and nutrients (Groschwitz and Hogan, 2009; Turner, 2009). The intestine is where various immune mechanisms contribute to pathogen defence. Toxins that alter the integrity of intestinal epithelium are likely to have consequences for both nutrient absorption and pathogen exclusion. Intestinal epithelial cells transport nutrients and fluids and serve to restrict the access for luminal antigens to the inter-

nal milieu. Any damage leads to enhanced permeability of the cell layer. There are few recent studies on the impact of dietary AFB₁ on gut function in relevant animal models (Grenier and Applegate, 2013). A common in vitro model system for gut integrity is the Caco-2 cell line (human epithelial colorectal cells). In this model, aflatoxin (150 µM) decreased trans-epithelial electrical resistance (Gratz et al., 2007).

Fumonisin

Two studies were conducted in BALB/c mice with five daily subcutaneous injections of 2.25 mg/kg bw fumonisin B₁ (FB₁). The FB₁ treatment resulted in an increase in the T-lymphocyte population in the spleen of female mice only, compared with controls (Johnson and Sharma, 2001). At a dose of 2.25 mg/kg bw, FB₁ dramatically reduced the immature CD4⁺/CD8⁺ double-positive cell population in the thymus of female mice but not of male mice (Johnson and Sharma, 2001). In a second study under the same conditions, FB₁ treatment markedly reduced relative spleen and thymus weights in female mice but not in male mice. Decreased plasma immunoglobulin G (IgG) levels were seen in female mice, and the effect was smaller in male mice. In addition, concanavalin A- and phytohaemagglutinin-induced T-lymphocyte proliferation was significantly reduced in female mice exposed to FB₁. The results of this study suggest that FB₁ is immunosuppressive in mice. The magnitude of the effect was highly dependent on sex; female mice were more susceptible than male mice (Johnson et al., 2001).

Fumonisin has been demonstrated to alter intestinal barrier integrity (Bouhet et al., 2004) and immune function in several studies that affected animal health. Other

effects on immune responses included alterations in cytokine expression, decreased antibody titre in response to vaccination, and increased susceptibility to secondary pathogens (Bulder et al., 2012). In swine, oral exposure to fumonisin resulted in sex-specific decreased antibody titres after vaccination and increased susceptibility to secondary pathogens (Oswald et al., 2003; Halloy et al., 2005; Marin et al., 2006). There is one study in swine exposed to pure fumonisin at a dose of 1.5 mg/kg bw for 7 days. FB₁ treatment induced a significant downregulation of the expression of IL-4 mRNA in the spleen and mesenteric lymph nodes (Taranu et al., 2005). Also, an extract of culture material containing fumonisin was incorporated in the basal diet to provide feed containing FB₁ at 8 mg/kg of feed for 28 days. The animals were immunized subcutaneously with Agavac, a vaccine made with a combination of formalin-inactivated *Mycoplasma agalactiae* strains, followed by a booster shot 2 weeks later. Exposure to the contaminated diet diminished the specific antibody titre after vaccination against *M. agalactiae*. In contrast, ingestion of the contaminated feed had no effect on the serum concentration of the immunoglobulin subsets (IgG, IgA, and IgM). The authors concluded that FB₁ altered the cytokine profile, which in turn affected the antibody response (Taranu et al., 2005).

In pigs fed a diet containing fumonisin at about 0.25 mg/kg bw, multifocal atrophy and villi fusion, apical necrosis of villi, cytoplasmic vacuolation of enterocytes, and oedema of lamina propria were observed in intestinal tissue compared with controls. Lymphatic vessel dilation and prominent lymphoid follicles were also observed (Bracarense et al., 2012).

No information was provided on the functional impact of these morphological changes. Modulation of intestinal cytokine production was also observed in pigs exposed to fumonisin, as well as in intestinal cell lines (Bouhet et al., 2006; Bra-careense et al., 2012). There have

been at least two studies in mice showing FB treatment-induced disruption of sphingolipid metabolism in the small intestine. One study used subcutaneous injection (single dose, 25 mg/kg bw) and the other oral gavage (single dose, 25 mg/kg bw) (Enongene et al., 2000, 2002). This

work illustrated the importance of sphingolipids and sphingolipid metabolites in the gut in relation to inflammation and barrier function, and also in the regulation of inflammatory response associated with endotoxin and microbial sepsis (Enongene et al., 2000, 2002).