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Aflatoxin B_1 interferes with the antigen-presenting capacity of porcine dendritic cells

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ABSTRACT

Aflatoxins (AFs) are harmful to animal and human health upon consumption of AF-contaminated feed or food. Among many forms of AFs, aflatoxin B_1 (AFB_1) is the most toxic and carcinogenic. In addition, AFB_1 impairs cell-mediated immunity, although the exact mechanism of this immunotoxicity is currently unknown. By far the most pivotal cells in the induction of immune responses are dendritic cells (DCs). These highly specialised cells dictate T-cell polarisation depending on the nature of the encountered antigens and environmental cues. To elucidate the effect of AFB_1 on the function of DCs, we used porcine monocyte-derived DCs (MoDCs) as a model system. A low dose of AFB_1 transiently reduced the phagocytic capacity of MoDCs. Furthermore, as compared to untreated MoDCs, AFB_1 significantly downregulated the cell surface expression of the co-stimulatory molecule CD40 at 12 h post treatment, while at 24 h the membrane expression levels of CD40 and the activation marker CD25 were significantly upregulated. Interestingly, the T-cell proliferation-inducing capacity of DCs was diminished upon AFB_1 treatment. In contrast, the cytokine secretion pattern of AFB_1-treated MoDCs was similar to mock-treated MoDCs. The results in this study indicate that a low level of AFB_1 dysregulates the antigen-presenting capacity of DCs, which could explain the observed immunotoxicity of this mycotoxin, and further stress the need to reduce AFB_1 levels in agricultural commodities.

1. Introduction

Mycotoxins, which are toxic secondary metabolites produced by molds, are potentially harmful to human and animal health upon consumption of mycotoxin-contaminated food or feed. The contamination of agricultural products by mycotoxin-producing molds remains a serious threat for animal and human health. The FAO estimated that 25% of the crops worldwide are contaminated by fungi and get affected by mycotoxins (CAST, 1989). As a consequence, many countries have put in place specific regulations for acceptable concentrations of mycotoxins in feed and/or food to address the safety concerns caused by these mycotoxins (Reddy et al., 2009). Despite monitoring programs, humans and animals could still be exposed to mycotoxins, present at low levels in feed and food. Among these mycotoxins, ochratoxin A, fumonisin B_1 and aflatoxins (AFs) are the most toxic to mammals. AFs are produced by fungi belonging to several *Aspergillus* species, mainly *A. flavus* and *A. parasiticus*, and can contaminate numerous agricultural products frequently used as feed- and foodstuffs (Whitlow and Hagler, 2004; Reddy et al., 2009; Bath et al., 2010; Marin et al., 2013). Among several identified AFs, aflatoxin B_1 (AFB_1) is the predominant form with the highest toxic potential. Similar to other mycotoxins, it possesses broad and nonselective toxic effects *in vivo* including teratogenic, mutagenic, hepatotoxic, cytotoxic and immunotoxic effects in both animals and humans (Wild and Turner, 2002; Oswald et al., 2003; Williams et al., 2004; Rawal et al., 2010; Chaytor et al., 2011). As a consequence, the International Agency for Research on Cancer (IARC) has classified AFB_1 as a group 1 carcinogen (IARC, 2003).

Upon consumption of AFB_1-contaminated feed, AFB_1 enters the body via passive diffusion through the gastro-intestinal epithelia due to its lipophilic nature. As the intestinal microbiota hardly degradate AFB_1, this enhances its bioavailability. As a consequence, AFB_1 rapidly appears in the blood circulation (Battacone et al., 2003; Masoero et al., 2007; Martins et al., 2007; Gallo et al., 2008). In the intestinal tissues and the liver this mycotoxin is then converted through the activity of cytochrome P450 enzyme family members and glutathione S-transferase to more or less toxic hydroxylated metabolites, such as AFM_1, AFQ_1 and aflatoxicol. Unmetabolized AFB_1 and its metabolites are then secreted via milk, urine and other secreta. Hence, the toxicity of AFB_1 depends not only on its intact form, but also on its biotransformation to reactive

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hydroxylated derivatives (Tulayakul et al., 2007; Ayed-Boussema et al., 2012; Gross-Steinmeyer and Eaton, 2012; Josse et al., 2012).

AFB_1 causes immunosuppression in domestic animals, such as poultry, pigs and ruminants, and humans upon chronic dietary exposure (Gong et al., 2004; Bondy and Petska, 2000; Chaytor et al., 2011; Marin et al., 2013). Indeed, ingestion of AFB_1-contaminated feed increases the susceptibility to infection and reduces vaccine-induced protection (Venturini et al., 1996; Meissonnier et al., 2008). AFB_1 mainly targets the innate and cell-mediated immunity, while humoral immune responses are largely unaffected, although this varies with the species and the AFB_1 dose (Meissonnier et al., 2006). Both natural killer cytolysis and several macrophage functions, such as phagocytosis, production of reactive oxygen species (ROS) and intracellular killing, are impaired by AFB_1 (Liu et al., 2002; Qureshi et al., 1998). In addition, this mycotoxin dysregulates neutrophil function in a bovine model, resulting in a decreased phagocytosis and intracellular ROS production (Mehrzad et al., 2011). Previous studies have demonstrated that the exposure of pigs to AFB_1-contaminated feed impairs the cell-mediated adaptive immune response upon vaccination, especially T-cell proliferation. This led to the hypothesis that the consumption of AFB_1-contaminated feed resulted in a decreased T-cell activation via its effects on dendritic cells (Meissonnier et al., 2008).

Dendritic cells (DCs) are key professional antigen-presenting cells (APCs) playing a crucial role by bridging innate and adaptive immunity. As such, DCs are pivotal in the induction of immune responses to control and eliminate pathogens (Banchereau et al., 2000). DCs are localised at peripheral tissues where they act as immune sentinels continuously patrolling and sampling environmental antigens (Mowat, 2003; Joffre et al., 2009). Upon antigen encounter and processing, DCs mature upregulating MHCII and costimulatory molecules to efficiently present antigen to naive T cells. Moreover, cytokines secretion pattern dictates the polarisation of naïve $CD4^+$ T-cells into effector Th1, Th2, Th17 or regulatory T cells, which in turn drive the ensuing immune response to protect the host from invading pathogens (Huang et al., 2001; de Jong et al., 2005; Shortman and Naik, 2007; Joffre et al., 2009).

The immunotoxic effects of low levels of AFB_1 on DCs are however still unclear. Since the outcome of every immune response is largely controlled by DCs, we hypothesised that a direct influence of AFB_1 on function of DCs might play a role in mediating the immunotoxic effects of AFB_1 in swine. To verify our hypothesis swine monocyte-derived DCs (MoDCs), a well-established DC model, were exposed to AFB_1 and their antigen-presenting capacities were assessed (Carrasco et al., 2001; Bimczok et al., 2007; Devriendt et al., 2010, 2013). Besides the sensitivity of pigs to AFB_1, they represent an important, economically relevant animal model, whose immune system in many aspects closely resembles that of humans, facilitating the extrapolation of experimental data to man (Fairbairn et al., 2011; Meurens et al., 2012).

2. Materials and methods

2.1. Aflatoxin B_1

Aflatoxin B_1 was obtained from Sigma (Sigma–Aldrich, Deisenhofen, Germany) and first dissolved in 96% ethanol (0.1 mg/ml) according to Mehrzad et al. (2011). Further dilutions were made with Dulbecco's PBS (Sigma–Aldrich). AFB_1 was then added to the cell cultures at a final concentration of 10 ng/ml. This AFB_1 concentration could reflect naturally-occurring levels in young piglets. Indeed, when animals are fed feedstuffs contaminated at the upper limit of 10 μg/kg feed on a dry matter (DM) basis which is tolerated by the EU feed legislation (2002) and considering the daily feed intake of piglets, the AFB_1 level in the blood stream could reach 5–10 ng/ml. Moreover, far higher levels than the selected dose may be encountered by animals in less affluent nations where mycotoxin monitoring programs are absent.

2.2. Generation of porcine monocyte-derived dendritic cells

Heparinised blood samples were obtained from the external jugular vein of Belgian Landrace pigs (8–20 weeks old) kept as blood donors under standard conditions at the Faculty of Veterinary Medicine, Merelbeke, Belgium. All animal experiments were in accordance with the local animal welfare regulations and were approved by the Ethical Committee of the Faculty of Veterinary Medicine.

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by lymphoprep density gradient centrifugation. Monocytes were further enriched to a purity of >95% by immunomagnetic bead selection (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) using the anti-CD172a mAb (clone 74-12-15a; Pescovitz et al., 1984) and goat anti-mouse microbeads together with LS separation columns (MACS, Miltenyi Biotec). $CD172a^+$ monocytes were cultured in 24-well plates (Nunc, Thermo Fisher Scientific, Langenselbold, Germany) at a density of 5.0×10^5 cells/ml in phenol-red free Dulbecco's modified Eagle's Medium (DMEM; Gibco, Merelbeke, Belgium), supplemented with 10% (v/v) FCS (Greiner Bio One, Wemmel, Belgium), 100 U/ml penicillin (Gibco), 100 μg/ml streptomycin (Gibco), recombinant porcine (rp) granulocyte–macrophage colony-stimulating factor (GM-CSF; Inumaru et al., 1998) and rpIL-4 (R&D systems, Abingdon, UK) and incubated at 37 °C in a humidified atmosphere at 5% CO_2 to generate MoDCs as previously described (Devriendt et al., 2010). On day 3 of the culture period, MoDCs were fed by addition of fresh medium supplemented with rpGM-CSF and rpIL-4 at the same concentrations. On day 4 or 5 of the culture period cells with long membrane protrusions, a typical feature of immature dendritic cells, dominated the cell culture (Fig. 1).

2.3. Phagocytosis assay

Immature MoDCs were stimulated with AFB_1 (10 ng/ml) for 1, 2, 12 and 24 h at 37 °C, 5% CO_2 and a humidified atmosphere. Next, 5.0×10^6 FITC-loaded polystyrene microparticles (1.0 μm, Sigma–Aldrich) were added to the stimulated MoDCs (10 microparticles/DC) and incubated for 3 h at 37 °C in a humidified atmosphere at 5% CO_2. Subsequently, the cells were harvested on

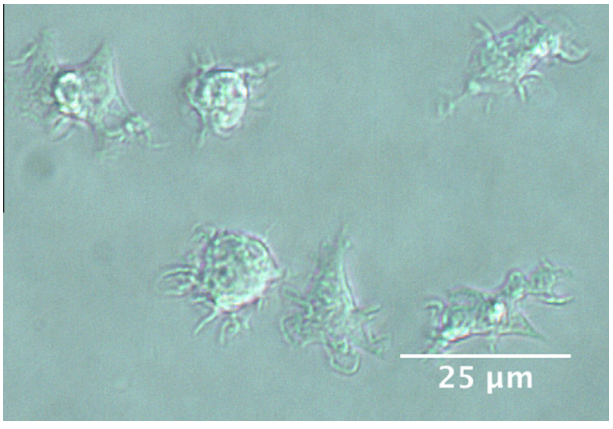

Fig. 1. A bright-field microscope image of porcine immature MoDCs at day 5 of culture. These cells possess long membrane protrusions or dendrites, a hallmark of immature dendritic cells.

ice, washed with ice-cold PBS and the internalisation of the microparticles was assessed by flow cytometry with a FACSCanto flow cytometer with a minimum event count of 20,000 and analysed with FACSDiva 6.1.3 software (BD biosciences, Erembodegem, Belgium). The results from this assay were presented as the percentage of phagocytosed microparticles relative to mock-stimulated MoDCs (control MoDCs). The phagocytotosis index was calculated with the following formula: phagocytotosis index = (phagocytosis by AFB_1-treated MoDCs/phagocytosis by control MoDCs) $*$ 100.

2.4. Cell surface expression of DC activation markers

The cell surface expression of DC activation markers upon stimulation of the MoDCs with medium and AFB_1 for 12 and 24 h was assessed by flow cytometry using mAbs against MHCII (MSA3, IgG_{2a}, Lunney et al., 1994), CD40 (G28-5, IgG_1, anti-human, Bimczok et al., 2007), CD25 (K231.3B2, IgG_1, Bailey et al., 1992) and a human CTLA4-muIgG_{2a} fusion protein (Ancell, Bayport, MN, USA), respectively, followed by R-phycoerythrin and AlexaFluor-647 conjugated isotype-specific anti-mouse secondary antibodies (Molecular Probes, Life Technologies, Merelbeke, Belgium). Human CTLA4 CD152) is able to bind to porcine CD80 and CD86 (Vaughan et al., 2000). Briefly, MoDCs were harvested and washed in staining medium (DMEM + 1% FCS) and then incubated with pre-titrated saturating concentrations of the primary Abs for 20 min at 4 °C. Cells stained with isotype-matched irrelevant mAbs (Molecular Probes) were used to assess aspecific binding. After washing, the cells were stained for 20 min at 4 °C with the secondary Abs in staining medium. Next, the cells were washed, propidium iodide (PI; 5 μg/ml) was added and data were acquired and analysed as described above. The relative marker expression (%) was calculated as: $\% = [(MFI_{treatment} - MFI_{control})/MFI_{control}] * 100$.

2.5. T-cell proliferation assay

The T-cell stimulatory capacity of MoDCs was analysed in an allogenic T-cell proliferation assay. T-lymphocytes were isolated from the PBMC fraction by enriching $CD6^+$ cells to a purity of >95% by positive immunomagnetic selection with the α-CD6 mAb (IgG_1, clone a38b2; Saalmüller et al., 1994) and goat anti-mouse microbeads together with LS columns (MACS, Miltenyi Biotech). MoDCs were treated with AFB_1 or left untreated for 12 and 24 h, thereafter the cells MoDCs were harvested, washed and counted. MoDCs were then co-cultured in triplicate at titrated numbers with 2.0×10^5 allogenic $CD6^+$ T-cells in round-bottomed 96-well microtiter plates (Nunc). $CD6^+$ T cells stimulated with 5 μg/ml ConA were used as a positive control. Cell cultures were maintained in DMEM, 10% FCS, penicillin/streptomycin and 2-mercapto-ethanol (50 μM) at 37 °C in a humidified atmosphere at 5% CO_2. After 5 days of culture, the cells were pulse-labelled with 1 μCi/well [^{3}H]methyl-thymidine (Amersham ICN, Bucks, UK) for another 18 h. Cells were harvested onto glass fibre filters (Perkin–Elmer, Life Science, Brussels, Belgium) and the [^{3}H] methyl-thymidine incorporation was measured using a β-scintillation counter (Perkin–Elmer). The data were presented as a stimulation index, which was calculated as followed: stimulation index = cpm cocultures $DCs_{treatment}$/cpm cocultures $DCs_{control}$.

2.6. Analysis of the cytokine secretion profile by ELISA

MoDCs were generated and stimulated with AFB_1 as mentioned above. After 24 h cell-free culture supernatant was collected and the porcine TNFα, IL-1β, IL-6, IL-8 and IL-10 cytokine concentrations were determined with commercially available ELISA kits (TNFα, IL-1β, IL-6 and IL-8: Duoset, R&D systems; IL-10: Life Technologies) according to the manufacturer's instructions. Briefly, microtiter plates were coated overnight at room temperature with capture antibody (Ab) specific for the analysed cytokines. The plates were washed, blocked and samples and standards were analysed in duplicate with the detection Ab and the streptavidin-HRP system. The samples were diluted ½ (TNFα, IL-1β, IL-6, IL-10) or ¼ (IL-8) in reagent diluent. Optical densities were measured in an ELISA plate reader at 450 nm. The cytokine concentrations were calculated using DeltaSOFT JV 2.1.2 software (BioMetallics, Princeton, NJ, USA) with a 4-parameter curve-fitting algorithm applied for standard curve calculations.

2.7. Statistical analysis

Statistical analyses were performed with SPSS 20. The effect of AFB_1 on the phagocytosis of microparticles, DC marker expression, T-cell proliferation and the cytokine secretion pattern were assessed with the nonparametric Mann–Whitney test. The significance level was set at $p < 0.05$.

3. Results

3.1. Aflatoxin B_1 affects the phagocytotic capacity of MoDCs

Previous studies have indicated that AFB_1 can decrease the phagocytotic activity of innate immune cells, such as neutrophils and macrophages (Moon et al., 1999; Mehrzad et al., 2011). As professional APCs, DCs are proficient in phagocytosis of encountered pathogens or other particulate matter. Here, we sought to assess the effect of AFB_1 on the DC-mediated phagocytosis of microparticles. MoDCs were treated at different time points with a low concentration of AFB_1 before the addition of the polystyrene microparticles. At this low concentration AFB_1 did not affect cell viability (data not shown). After 1 h stimulation AFB_1 significantly increased the internalisation of polystyrene microparticles by DCs ($p = 0.037$), while at later time points (2 and 12 h) this phagocytotic activity decreased significantly as compared to the 1 h time point and to mock-treated MoDCs (12 h: $p = 0.037$) (Fig. 2). Remarkably, at 24 h AFB_1-treated DCs were again able to phagocytose particulate matter, indicating that the effect is either transient or that DCs can counter the toxic effects of AFB_1.

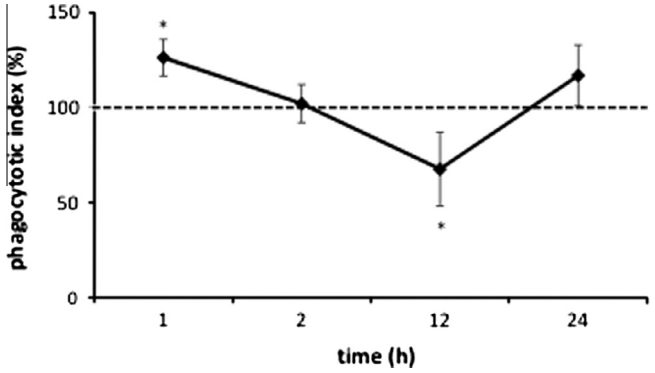

Fig. 2. Aflatoxin B_1 (AFB_1) affects the phagocytotic activity of porcine monocyte-derived dendritic cells (MoDCs). MoDCs were stimulated at different time points with 10 ng/ml AFB_1 as indicated in the *x*-axis. Fluorescent microparticles (1.0 μm) were added to the MoDCs to assess the effect of AFB_1 treatment on their phagocytotic capacity. The dashed line represents the phagocytotic index of non-AFB1 treated MoDCs, while the full line represents the phagocytotic index of AFB_1-treated MoDCs. The data are presented as the mean% ± sem ($n = 3$). $^*p < 0.05$ to untreated cells.

3.2. Aflatoxin B_1 enhances DC activation marker expression

Besides antigen uptake DCs also need to upregulate the cell surface expression of peptide-MHC complexes and costimulatory molecules to efficiently present processed antigens to naive T-cells. Thus, in addition to phagocytosis, we wanted to elucidate the effect of AFB_1 on the activation or phenotypical maturation of porcine MoDCs by assessing the expression of the cell surface markers MHCII, CD40, CD80/86 and CD25 (=IL-2R). The latter is considered as an important marker for porcine DC activation (Pilon et al., 2009; Devriendt et al., 2013). As indicated in Fig. 3, AFB_1 treatment had a clear time-dependent effect on the cell surface expression of these markers. After 12 h of AFB_1 treatment, all assayed markers were decreased as compared to control DCs, although this was only significant for CD40 ($p = 0.037$) (Fig. 3). In contrast, upon 24 h stimulation with AFB_1, both CD40 and CD25 expression were significantly upregulated as compared to untreated MoDCs ($p = 0.014$), indicating a phenotypical DC maturation or at least activation of these AFB_1-treated DCs.

3.3. Aflatoxin B_1 reduces the T-cell stimulatory activity of MoDCs

To further assess if the observed AFB_1-mediated phenotypical DC activation correlates with a functional activation, the effect of AFB_1 on the ability of DCs to induce T-cell proliferation was investigated. In contrast to the phenotypical DC activation, AFB_1 failed to enhance the T-cell stimulatory capacity of DCs (Fig. 4). Indeed, while MoDCs stimulated for 12 h with AFB_1 they did not differ in their T-cell stimulatory ability in comparison with immature MoDCs; treating MoDCs for 24 h with AFB_1 significantly decreased their ability to induce T-cell proliferation ($p = 0.002$).

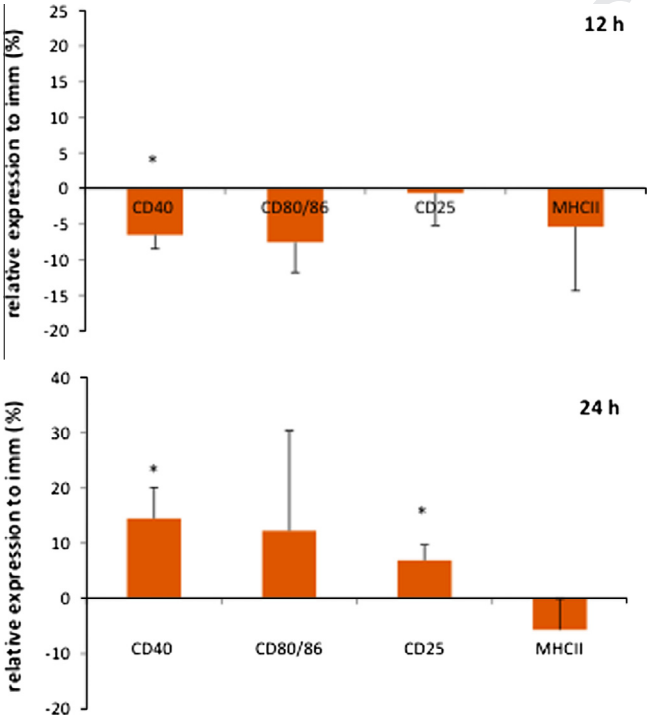

Fig. 3. AFB_1 affects the cell surface expression of DC activation markers. AFB_1-treated MoDCs (12 and 24 h) were analysed for their expression of CD40, CD80/86, CD25 and MHCII by flow cytometry. The data are presented as the mean (±sem) relative increase as compared to untreated MoDCs ($n = 4$). $^{*}p < 0.05$ to untreated cells. imm = immature (untreated or control) MoDCs.

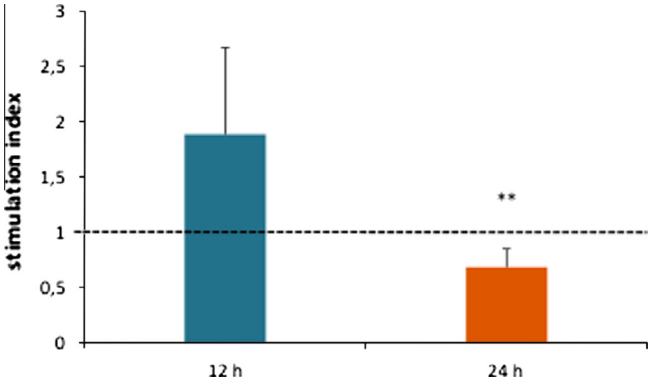

Fig. 4. AFB_1 fails to enhance the T-cell stimulatory capacity of porcine monocyte-derived dendritic cells. MoDCs were treated with 10 ng/ml AFB_1 for 12 and 24 h. AFB_1-treated MoDCs were subsequently co-cultured with 2.0×10^5 $CD6^+$ T cells at a DC/T ratio of 1:30. T-cell proliferation was measured via ^3H thymidin incorporation. Data are represented as the mean stimulation index ± sem ($n = 4$). $^{**}p < 0.01$ to untreated cells.

3.4. Aflatoxin B_1 does not impair cytokine secretion by MoDCs

As AFB_1 decreases the ability of DCs to activate T-cells and as cytokines play a crucial role in the activation and polarisation of T-cells, we next sought to determine the effect of aflatoxin B_1 on the DC cytokine secretion profile. As shown in Fig. 5, we observed a small increase in IL-1β and IL-6 secretion by DCs due to the presence of AFB_1 for 12 and 24 h, respectively, although these increased levels were not significantly different from the IL-6 and IL-1β secretion levels of immature MoDCs. The IL-10, IL-8 and TNFα secretion levels were unaffected by AFB_1 treatment of MoDCs (Fig. 5).

4. Discussion

Recent data indicate that the chronic exposure of piglets to low mycotoxin levels, such as fumonisin B_1 (FB_1), still could affect the intestinal immune system and prolong infection with intestinal pathogens (Oswald et al., 2003; Devriendt et al., 2009). Similar to FB_1, AFB_1 in feed causes immunotoxicity and interferes with vaccination efficacy in piglets. Indeed, ingestion of AFB_1-contaminated feed by piglets resulted in a reduced T-cell proliferation, presumably due to a direct effect of AFB_1 on DCs (Meissonnier et al., 2008). Basically, T-cell activation by DCs requires three interrelated signals: (1) the interaction of the T-cell receptor with peptide-MHC complexes on the DC surface, (2) DCs have to upregulate the expression of co-stimulatory molecules, such as CD40 and CD80/86 to fully activate naïve T-cells and (3) cytokine secretion which will influence the polarisation of the activated T-cells. If one of those signals is absent, T-cell activation and consequently T-cell proliferation will be impaired (Mowat, 2003). As DCs are the most potent APCs with the unique ability to activate naïve T-cells, we aimed to analyse the effect of a low level of AFB_1 on the function of DCs. To the best of our knowledge, this is the first study elucidating the effects of AFB_1 on DC function in piglets.

Central to the function of DCs is their ability to take up antigens. Previous studies have indicated that AFB_1 can decrease the phagocytotic activity of innate immune cells, such as neutrophils and macrophages (Moon et al., 1999; Mehrzad et al., 2011). Our results show that AFB_1 both enhances and inhibits the phagocytic capacity of MoDCs in a time-dependent manner. This fluctuating effect of AFB_1 on the uptake of microparticles by MoDCs is intriguing. The initial rise in the phagocytosis of microparticles by MoDCs at 1 h

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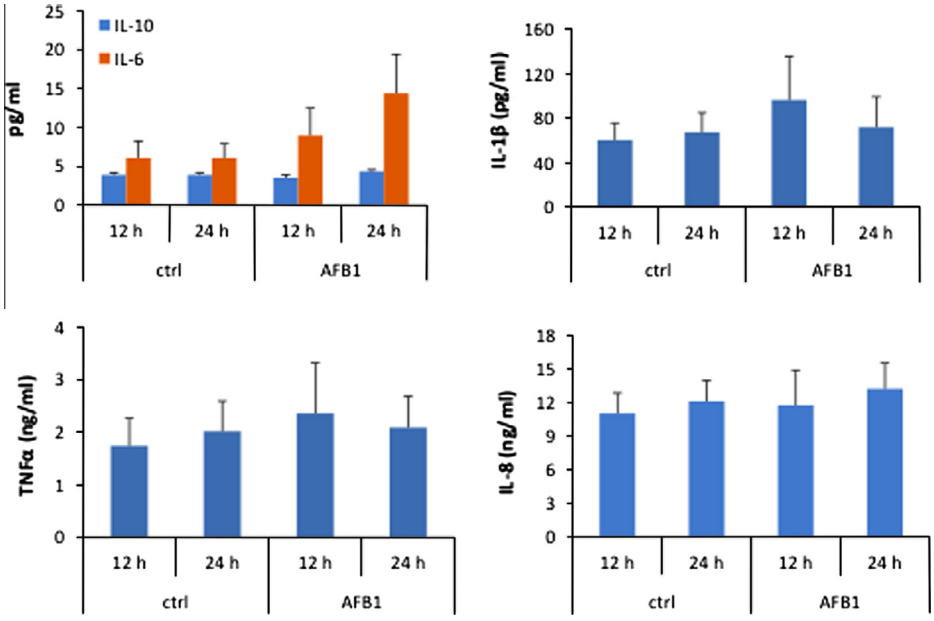

Fig. 5. Cytokine expression pattern of MoDCs treated with 10 ng/ml and 0 ng/ml (cntrl) of AFB1 and analysed with ELISA assays. Though non-significant, AFB1 induced a higher IL-6 secretion by MoDCs upon 24 h incubation. In contrast to IL-6, the secretion levels of IL-10, IL-1β, IL-8 and TNF-α were unaffected by treatment of MoDCs with AFB_1. Data are represented as the mean ± sem ($n = 4$).

probably reflects a response of the DCs to mycotoxin sensing via the aryl hydrocarbon receptor (AhR). This ligand-dependent nuclear receptor is expressed by DCs and is involved in the detection of environmental pollutants, such as planar aromatic hydrocarbons (Gu et al., 2000; Hauben et al., 2008). In contrast to the elevated phagocytosis at early time points, the observed decrease in phagocytosis of microparticles by MoDCs at 12 h of AFB_1 treatment presumably indicates an AFB_1-induced maturation of the MoDCs or an interference of AFB_1 with the phagocytotic machinery of MoDCs. Indeed, AFB_1 interferes with membrane and actin cytoskeleton dynamics, two well-known key mechanisms necessary for efficient phagocytosis (Goodridge et al., 2012). Remarkably, at 24 h AFB_1-treated MoDCs were again able to phagocytose microparticles ($p = 0.05$ as compared to 12 h time point), indicating that the toxic effect is either transient or that MoDCs can counter the toxic effects of AFB_1. Interestingly, recent data suggests that AFB_1 upregulates AhR mRNA expression in human hepatocytes (Ayed-Boussema et al., 2012). In addition, activation of the AhR signaling pathway was detected by RNA-seq in the liver of AFB_1-treated rats (Merrick et al., 2013). The main downstream targets of AhR are the xenobiotic metabolizing monooxygenases CYP1A1 and CYP1A2, involved in the detoxification of AFB_1 (Stockinger et al., 2011; Ayed-Boussema et al., 2012). Based on these findings we propose that upon aflatoxin B_1 sensing via AhR DCs upregulate the expression of these detoxifying enzymes to counteract the toxic effect of AFB_1.

Similar to the phagocytosis assay, we observed a time-dependent effect of AFB_1 on the expression of MHCII, CD25 and the costimulatory molecules CD40 and CD80/86. Indeed, at 12 h it decreased as compared to mock-treated MoDCs, while at 24 h CD40 and the DC activation marker CD25 were remarkably upregulated, indicating a phenotypical maturation or at least activation of these AFB_1-treated MoDCs. Intriguingly, MHCII expression remained downregulated, hinting at an effect of AFB_1 on MHCII surface expression.

We observed that a low level of AFB_1 failed to enhance the T-cell stimulatory capacity of DCs and even diminished the T-cell proliferation-inducing capacity of MoDCs, which could explain the observed diminished cell-mediated immunity in AFB_1-fed piglets observed by Meissonnier et al. (2008). This decreased T-cell proliferation-inducing activity might be due to the effects of AFB_1 on the secretion of cytokines. However, the analysis of the DCs cytokine secretion profile indicated that increased IL-10 secretion levels, which are able to suppress the proliferation of effector T-cells, can be ruled out as a cause of the observed poor T-cell stimulatory capacity of AFB_1-treated MoDCs. Besides IL-10, TGFβ can also suppress T-cell proliferation through the induction of tolerogenic DCs and Tregs. Unfortunately, porcine MoDCs do not produce TGFβ and consequently we were unable to assess the effect of AFB_1 on TGFβ secretion by DCs (Li and Flavell, 2008; Saurer et al., 2007). The decreased T-cell stimulatory capacity of AFB_1-treated MoDCs also does not seem to be the result of decreased levels of pro-inflammatory cytokines as AFB_1 treatment did not affect the pro-inflammatory cytokine secretion profile of porcine DCs. This is in line with previous data as AFB_1 did not modulate IL-1β and TNFα expression in swine alveolar macrophages (Liu et al., 2002). Nonetheless, we assayed only a limited amount of cytokines and a potential effect of AFB_1 on other cytokines or chemokines cannot be excluded. Because the low level of AFB_1 did not affect the pro- and anti-inflammatory cytokine secretion pattern of DCs, one critical unanswered question is how AFB_1-treated MoDCs mediate the decreased T-cell proliferation. To our best knowledge the decreased T-cell proliferation could be the result of the AFB_1-mediated downregulation of MHCII surface expression, although this downregulation was not significant. Alternatively, activation of AhR by exo- and endogenous ligands induces tolerogenic DCs, which in turn promote $Foxp3^+$ Treg differentiation in a retinoic acid-dependent manner (Quintana et al., 2010; Stockinger et al., 2011; Weiner et al., 2011). Further research is warranted to investigate if AFB_1 can activate a similar mechanism in porcine DCs.

In conclusion, our novel finding demonstrates that even a low level of AFB_1 dysregulates the antigen-presenting functions of porcine DCs. Further functional assays are in progress to elucidate the molecular mechanisms behind this phenomenon. These results could explain at least in part the immunosuppressive effects of AFB_1 in animal models and humans and further stresses the need to reduce AFB_1 levels in feed and food.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Transparency documents associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tiv.2013.11.015.

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