Food and Chemical Toxicology 173 (2023) 113596

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Maternal dietary exposure to mycotoxin aflatoxin B_1 promotes intestinal immune alterations and microbiota modifications increasing infection susceptibility in mouse offspring

Patricia Bastos-Amador [a,b], Elsa Leclerc Duarte [c,d], Júlio Torres [e], Ana Teresa Caldeira [f], Inês Silva [c,d,f], Cátia Salvador [f], Ricardo Assunção [g,h], Paula Alvito [a,h], Manuela Ferreira [b,e,*]

[a] Food and Nutrition Department, National Institute of Health Dr. Ricardo Jorge, 1649-016, Lisbon, Portugal
[b] Champalimaud Foundation, Champalimaud Centre for the Unknown, 1400-038, Lisbon, Portugal
[c] University of Évora, School of Science and Technology, 7000-671, Évora, Portugal
[d] MED-Mediterranean Institute for Agriculture, Environment and Development, 7006-554, Évora, Portugal
[e] University of Coimbra, Center for Innovative Biomedicine and Biotechnology, Center for Neuroscience and Cell Biology, 3004-504, Coimbra, Portugal
[f] HERCULES Laboratory, Universidade de Évora, 7000-809, Évora, Portugal
[g] IUEM, Instituto Universitário Egas Moniz, Egas Moniz-Cooperativa de Ensino Superior, CRL, 2829 - 511, Caparica, Portugal
[h] University of Aveiro, CESAM - Centre for Environmental and Marine Studies, 3810-193, Aveiro, Portugal

ARTICLE INFO

Handling Editor: Dr. Bryan Delaney

Keywords:
Mycotoxins
Early-life
Maternal dietary exposure
Intestinal immune system
Microbiota
Infection

ABSTRACT

Mycotoxins are secondary metabolites produced by fungi occurring in food that are toxic to animals and humans. Early-life mycotoxins exposure has been linked to diverse pathologies. However, how maternal exposure to mycotoxins impacts on the intestinal barrier function of progeny has not been explored. Here, exposure of pregnant and lactating C57Bl/6J female mice to aflatoxin B_1 (AFB_1; 400 μg/kg body weight/day; 3 times a week) in gelatine pellets, from embryonic day (E)11.5 until weaning (postnatal day 21), led to gut immunological changes in progeny. The results showed an overall increase of lymphocyte number in intestine, a reduction of expression of epithelial genes related to microbial defence, as well as a decrease in cytokine production by intestinal type 2 innate lymphoid cells (ILC2). While susceptibility to chemically induced colitis was not worsened, immune alterations were associated with changes in gut microbiota and with a higher vulnerability to infection by the protozoan *Eimeria vermiformis* at early-life. Together these results show that maternal dietary exposure to AFB_1 can dampen intestinal barrier homeostasis in offspring decreasing their capability to tackle intestinal pathogens. These data provide insights to understand AFB_1 potential harmfulness in early-life health in the context of intestinal infections.

1. Introduction

Lifetime between conception and breastfeeding encloses the most critical period of development and lays the foundation of future individual's health (Godfrey et al., 2010). The intestinal mucosa, a body surface separating the core of the organism from the external world, undergoes critical developmental processes, including concomitantly establishment of the intestinal immune system and microbiota (De Agüero et al., 2016; Stras et al., 2019; Torow et al., 2016). Exposure to harmful compounds present in foods, as mycotoxins, during early-life may potentially lead to intestinal immune developmental alterations and increase susceptibility to disease.

Mycotoxins are natural fungal secondary metabolites that contaminate food commodities (Al-Jaal et al., 2019; Boevre et al., 2015; Gong et al., 2016; Kachapulula et al., 2017; Magan and Olsen, 2004) and can have significant impact on health, including adverse pregnancy outcomes, foetal growth impairment and developmental defects (Alvito & Pereira-Da-silva, 2022; Assunção et al., 2016, 2019; Bryden, 2007; Guerre, 2020; S. H. Park et al., 2015; Payros et al., 2016; Shuaib et al., 2010). Poor agricultural practices and improper storage conditions are suitable for mycotoxin formation (Al-Jaal et al., 2019; Gong et al., 2016; Kachapulula et al., 2017). Mycotoxicosis occurrences have been recorded four times in Kenya from 2004 to 2014, with near to 211 deaths (Awuor et al., 2017) and additional outbreaks are foreseen. Despite legal

* Corresponding author. Champalimaud Foundation, Champalimaud Centre for the Unknown, 1400-038, Lisbon, Portugal.
E-mail address: manuela.ferreira@cnc.uc.pt (M. Ferreira).

https://doi.org/10.1016/j.fct.2022.113596
Received 13 October 2022; Received in revised form 19 December 2022; Accepted 28 December 2022
Available online 2 January 2023
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limits and governmental health control strategies to restraint exposure, mycotoxins are expected to be a growing food safety issue in the forthcoming years due to climate change (Battilani et al., 2016; Duchenne-Moutien and Neetoo, 2021), raising the risk for vulnerable groups, such as women and children (Andrews-Trevino et al., 2020; Assunção et al., 2018; T. Chen et al., 2022; Martins et al., 2018; Raiola et al., 2015; Tesfamariam et al., 2022).

One of the most hazardous mycotoxins is aflatoxin B_1 (AFB_1), which can be present in a variety of cereals, legumes and oilseeds (Alshannaq and Yu, 2017; Buszewska-Forajta, 2020; Eskola et al., 2020; Jallow et al., 2021) and has been classified as Group 1 by the International Agency for Research on Cancer (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012).

After ingestion, AFB_1 is quickly absorbed in the gastrointestinal tract (Hernández and E, 1996) being mainly metabolized in the liver to toxic compounds and reaching several internal organs (Al-Jaal et al., 2019; L. Wang et al., 2022). In pregnant females, AFB_1 metabolites can cross the placental barrier (El-Nahla et al., 2013; Partanen et al., 2009; Wangikar and Dwivedi, 2005) and the AFB_1 metabolite, AFM_1, has been detected in breast milk (Hsieh et al., 1993; Jafari et al., 2017; Lamplugh et al., 1988). The detection of AFB_1-lysine adducts in cord blood have demonstrated that the foetus have the capacity to convert aflatoxin into toxicologically active compounds and their detection in the infant children illustrates exposure over the first 1000 days of life (Groopman et al., 2014), raising serious health risk to young individuals. AFB_1 exposure during early-life leads to adverse birth outcomes and postnatal growth problems, including immune consequences (Alvito & Pereira-Da-silva, 2022; Augusto et al., 2021; Lauer et al., 2019; Tesfamariam et al., 2022).

Besides recognized as a hepatocarcinogen (Wogan, 1973), AFB_1 has well documented immune system modulation properties (Rushing and Selim, 2019). Ingestion of AFB_1-contaminated feeds increases susceptibility to infection (Joens et al., 1981; Venturini et al., 1996) and decreases protection conferred by vaccination (G. M. Meissonnier et al., 2008; Venturini et al., 1990). AFB_1 reduces cell-mediated immunity (Ghosh et al., 1991; G. Meissonnier et al., 2006); leads to reduced T cell responses (Scott et al., 1991); inhibits lymphocyte blastogenesis (G. M. Meissonnier et al., 2008); lymphocyte number alteration (Bondy and Pestka, 2000; He et al., 2014; He et al., 2014); and alters leukocyte activity and cytokine expression (Jiang et al., 2015; Methenitou et al., 2001; Silvotti et al., 1997). In addition, alterations in gut microbiota upon adulthood AFB_1 exposure have also been observed (Grosu et al., 2019; Ishikawa et al., 2017).

Accordingly, AFB_1 exposure during embryonic development or perinatal period can also negatively affect the immune system. Zebrafish embryos exposed to AFB_1 displayed increased neutrophil number and upregulation of inflammatory gene expression (Ivanovics et al., 2021). Direct administration of AFB_1 into chick embryos caused cytotoxicity in B and T lymphocytes (Dietert et al., 1983; Potchinsky and Bloom, 1993) and decreased cell-mediated immunity (Dietert et al., 1985; Neldon-Ortiz and Qureshi, 1992). Also exposure of broiler breeder hens to AFB_1-containing dietary resulted in suppression of humoral and lower macrophage activity in progeny (Qureshi et al., 1998). Some studies, show thymic defects and impairment of peripheral immune efficiency on weanling mammals directly treated with AFB_1 (Harvey et al., 1988; Panangala et al., 1986; Raisuddin et al., 1990; Reddy et al., 1987) or from mothers that were fed with AFB_1-containing diets (Mocchegiani et al., 1998; Silvotti et al., 1997). However, how maternal AFB_1 exposure during gestation and lactation impacts on intestinal immune system of the progeny remains largely unexplored.

The small intestine allows absorption of nutrients from food, while comprising a sophisticated immune system, which protects the core of the body from external assaults. Intestinal immune system development initiates *in utero* and continues during postnatal period concomitantly with microbiota formation (De Agüero et al., 2016; Stras et al., 2019; Torow et al., 2016). Despite their developmentally regulated genetic program, immune cells are shaped by environmental cues (M. Ferreira and Veiga-Fernandes, 2014; Goverse et al., 2016; Van De Pavert et al., 2014; Xu et al., 2015). Recently, we showed that prenatal and post-natal dietary micronutrient levels control immune system development, including intestinal lymphoid organ formation, and pre-sets offspring long term immunity (Goverse et al., 2016; Van De Pavert et al., 2014). Altogether, these evidences suggest that maternal dietary AFB_1-exposure may impact the development of the gut immune system and microbiota of the progeny.

In this study, we analysed intestinal lymphoid organs, immune cell populations, and the microbiota of offspring mice from mothers that were fed with AFB_1-containing diet during pregnancy and breastfeeding period. The progeny of these females revealed alterations of intestinal lymphocyte subsets, as well as changes in epithelia and microbiota composition upon maternal AFB_1 exposure. In this context, the predisposition to intestinal inflammation and infection was investigated in order to assess the mucosal barrier function in AFB_1-exposed offspring. This study provides insights on how AFB_1 exposure during early-life impacts intestinal immune development and infectious disease susceptibility.

2. Materials and methods

2.1. Mice

C57BL/6J mice were bought from The Jackson Laboratory. Mice were used at 8–14 weeks old. Males and females were bred at Champalimaud Foundation (FC) animal facility under specific pathogen-free conditions, maintained at 22 °C, with a 40% relative humidity, and a 12-h light/dark cycle. Standard rodent chow and filtered water were available *ad libitum*. All animal experiments were approved by national and local Animal Welfare Body (AWB), respectively, Direção Geral de Veterinária and FC ethical committees.

2.2. Aflatoxin B_1 and experiment design

Standard Aflatoxin B_1 (AFB_1; CAS Number: 1162-65-8) from *Aspergillus flavus* (purity >98%) was obtained from Sigma (St. Louis, MO) and a stock solution was prepared in acetonitrile at 5 mg/mL. For the experiments, males and females C57BL/6J were mated overnight and the day of vaginal plug detection was marked as embryonic day (E) 0.5. The weight of females was monitored at E11.5 to confirm pregnancy. Pregnant females were randomly assigned to 2 groups of at least 3 animals per group and treated 3 days a week with gelatine pellets containing 5 μg of AFB_1 (per animal per day) - dose designed "200 μg/kg body weight" of the animal; 9.2–12 μg of AFB_1 (per animal per day) – dose designed "400 μg/kg body weight" of the animal; or vehicle (dimethyl sulfoxide (DMSO; Figs. 1–5) or acetonitrile (50 μg/mL; Figs. 6 and 7). Pregnant females were treated since the embryonic day (E) 11.5 until weaning of the progeny. At least 2 independent experiments were performed for each result presented at 3. Section. The AFB_1 dose (9.2–12 μg per animal per day) was selected based on the chronic AFB_1 treatments used in other studies involving animals (Fetaih et al., 2014; Ishikawa et al., 2017; Jha et al., 2013), and the median lethal dose (LD50) value for male mice (ranging from 9 to 60 mg of AFB_1/kg body weight) (Almeida et al., 1996). The consumption of foodstuffs commonly contaminated with AFB_1 and the range of AFB_1 levels commonly found in foodstuffs consumed daily by the human population, especially in developing countries (Filazi and Sireli, 2013; Kamika and Takoy, 2011; Williams et al., 2004) were taken into consideration. Also, aflatoxicosis cases were reported occurring at similar or higher consumption of AFB_1 (Azziz-Baumgartner et al., 2005; Williams et al., 2004).

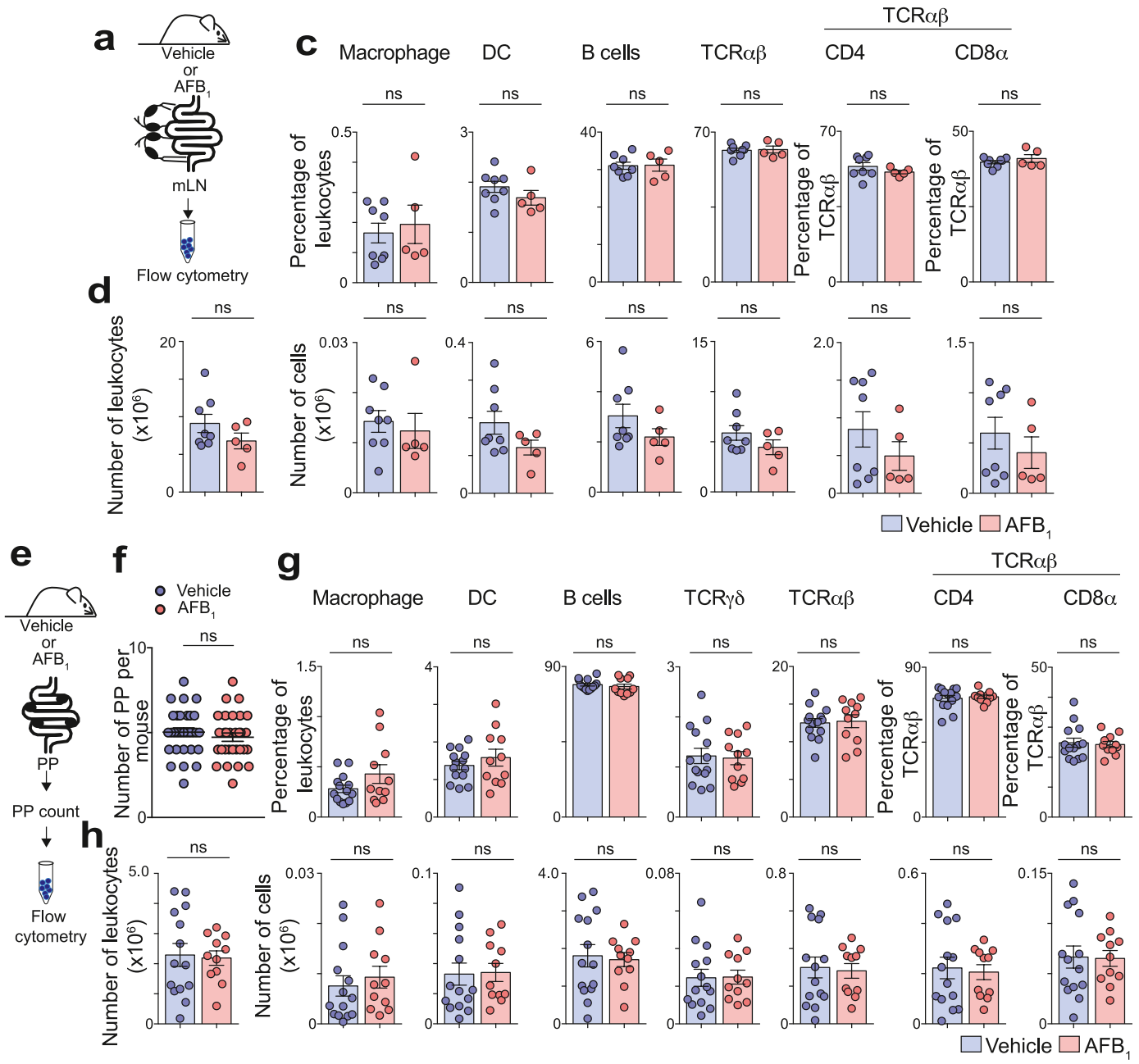

Fig. 1. Mesenteric lymph nodes and Peyer's patches of maternal AFB_1-exposed offspring. a. Schematic representation of mesenteric lymph node (mLN) analysis procedure. **c-d.** Frequency (**c**) and absolute number (**d**) of leukocytes in the mLN. Vehicle, n = 14; AFB_1, n = 11. **e.** Schematic representation of Peyer's patches (PP) analysis procedure. **f.** Number of PP per intestine. Vehicle and AFB_1, n = 30. **g-h.** Frequency (g) and absolute number (**h**) of leukocytes in PP. Vehicle, n = 14; AFB_1, n = 11. Data represent summary of at least 2 independent experiments **(c-d, f)** and 2 independent analyses (**g-h**). Each experiment includes at least 2 litters per condition (vehicle or AFB_1). With the exception of **b.**, each symbol is an individual mouse, n represents individual animals. Bars depict mean, error bars represent ± s.e.m. For statistical analysis, a two-tailed Mann–Whitney *U* test was used. ns, not significant.

2.3. Cell isolation

Isolation of small intestine lamina propria cells was as previously described (Qiu and Sheridan, 2018). In brief, intestines were thoroughly rinsed with cold PBS (1X), Peyer's patches were removed from the small intestine, and intestines were cut into 1 cm pieces and shaken twice for 15 min in HBSS containing 5% FBS, 10 mM Hepes, and 1 mM DTT. Epithelial cells were collected by incubating intestinal pieces in pre-warmed EDTA 5 mM solution for 15 min in the shaker at 180–200 rpm. After washing with HBSS with calcium and magnesium, the remaining intestinal pieces were digested in RPMI plus Liberase 50 μg/ml and DNaseI 100 μg/ml. Lamina propria leukocytes were purified by centrifugation for 25 min at 1200 g in a 40/80 Percoll (GE Healthcare) gradient.

2.4. Flow cytometry analysis and cell sorting

Cells suspensions were incubated with FcγR before antibody staining. For cytokine analysis *ex vivo*, cells were incubated with PMA (phorbol 12-myristate 13-acetate; 50 ng/ml) and ionomycin (500 ng/ml) (Sigma-Aldrich) in the presence of brefeldin A (eBioscience) for 4 h before intracellular staining. Intracellular staining for cytokines and transcription factors analysis was performed using IC fixation and Staining Buffer Set (eBioscience). Cell sorting was performed using

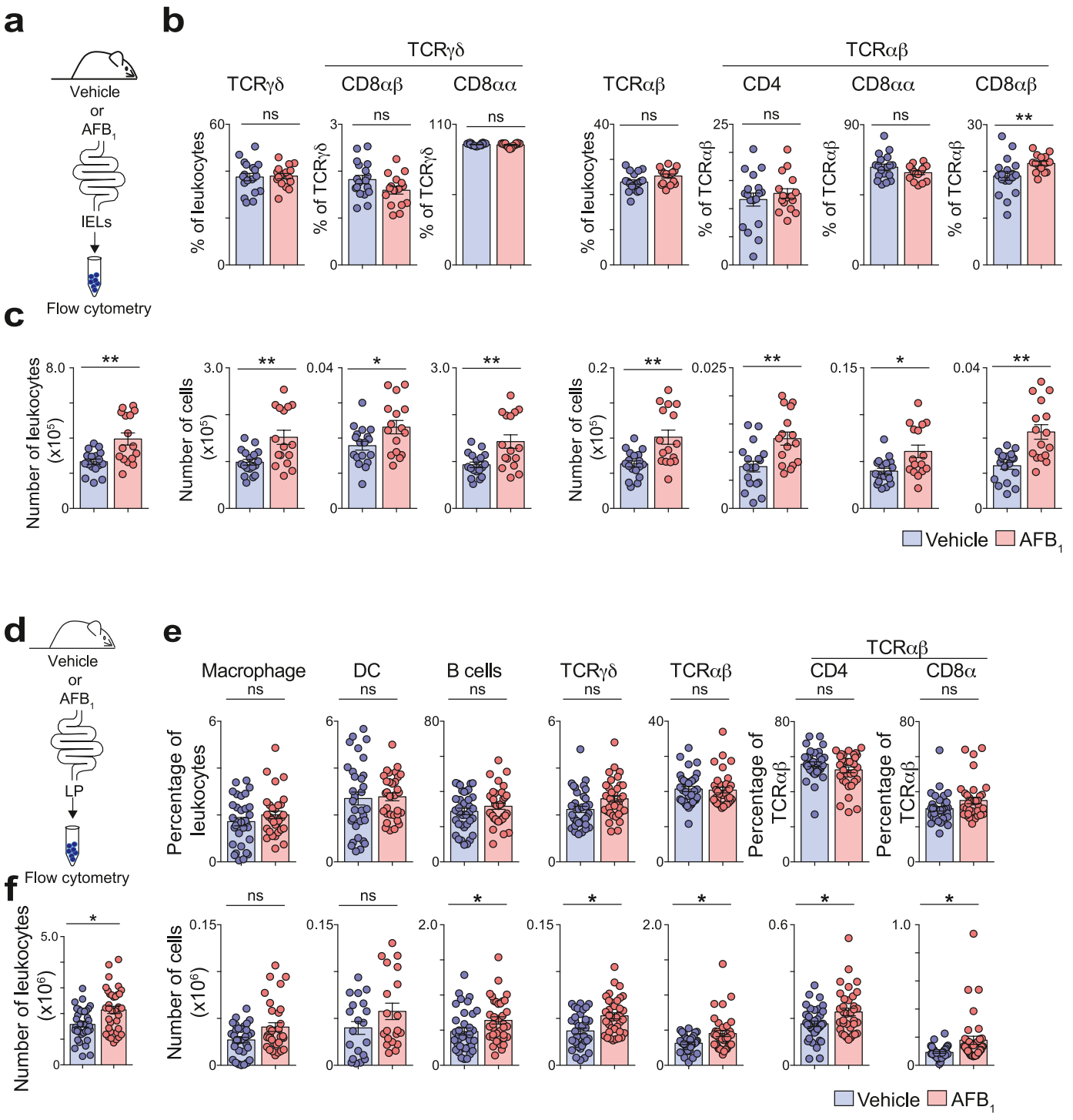

Fig. 2. Maternal AFB_1 exposure leads to increase of the number of adaptive lymphocytes in the lamina propria and epithelial compartment. a. Schematic representation of intraepithelial lymphocyte (IEL) compartment analysis procedure. **b-c.** Frequency (**b**) and absolute number (**c**) of IEL subsets. Vehicle, n = 18; AFB_1, n = 16. Mother females were treated with AFB_1 200 μg/kg body weight of the animal. **d.** Schematic representation of lamina propria (LP) compartment analysis procedure. **e-f.** Frequency (**e**) and absolute number (**f**) of LP leukocytes subsets. Vehicle and AFB_1, n = 20 to 36. Mother females were treated with AFB_1 400 μg/kg body weight. Data represent summary of 3 independent experiments (**e-f**) and 3 independent analyses (**b-c**). Each experiment includes 3 to 6 litters per condition (vehicle or AFB_1). Each symbol is an individual mouse, n represents individual animals. Bars depict mean, error bars represent ± s.e.m. For statistical analysis, a two-tailed Mann–Whitney *U* test was used. *P < 0.05; **P < 0.01; ns, not significant.

FACSFusion (BD Biosciences). Sorted populations were >95% pure. Flow cytometry analysis was performed on LSRFortessa X-20 (BD Biosciences). Data was analysed using FlowJo 10.5.0 software (Tree Star). LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen) was used in all samples to exclude dead cells. Cell populations were gated in live cells, both for sorting and flow cytometry analysis. Absolute numbers of leukocytes populations were quantified using Perfect Count Microspheres (Cytognos).

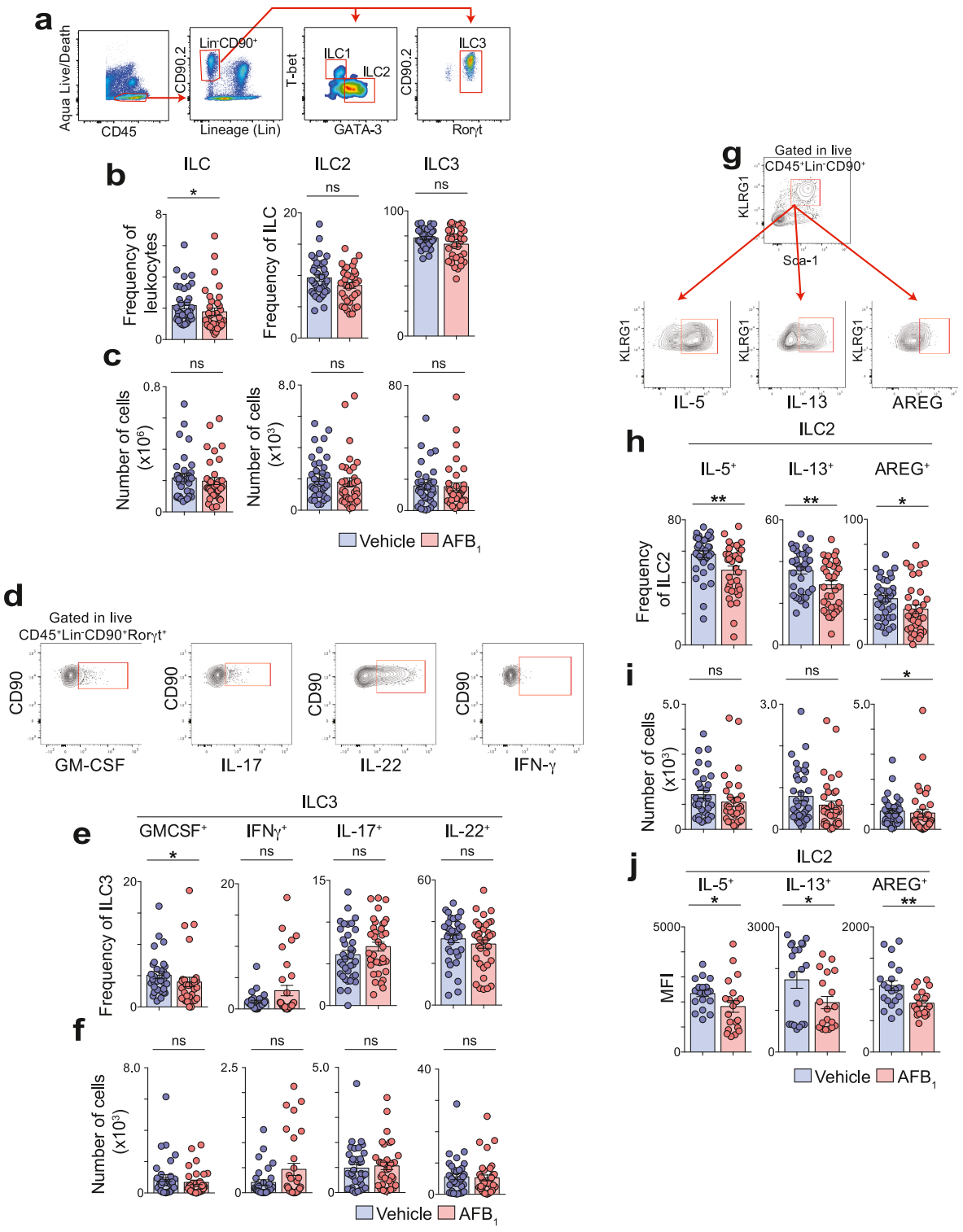

Fig. 3. Maternal AFB_1 exposure decreases cytokine production by ILC2 subsets. a. Representative flow cytometry plots showing gate strategy to identify innate lymphoid cell (ILC) groups 1, 2 and 3 using cell surface markers. Mother females were treated with AFB_1 400 μg/kg body weight. **b-c.** Frequency (**b**) and absolute number (**c**) of indicated ILC subsets. Vehicle and AFB_1, n = 36. **d.** Representative flow cytometry plots showing gate strategy to identify cytokine-expressing ILC3 subsets. **e-f.** Frequency (**e**) and absolute number (**f**) of indicated ILC3 subsets. Vehicle, n = 31 to 36; AFB_1, n = 30 to 35. **g.** Representative flow cytometry plots showing gate strategy to identify cytokine-expressing ILC2 subsets. **h-j.** Frequency (**h**), absolute number (**i**) and median fluorescence intensity (MFI) of indicated cytokine gated in total ILC2 subset. Vehicle and AFB_1, n = 32 to 36. Data represents summary of 3 independent experiments, which include 8 litters per condition of vehicle or AFB_1. Bars depict mean, each symbol is an individual mouse, error bars represent ± s.e.m. For statistical analysis, a two-tailed Mann–Whitney *U* test was used. *P < 0.05; **P < 0.01; ns, not significant.

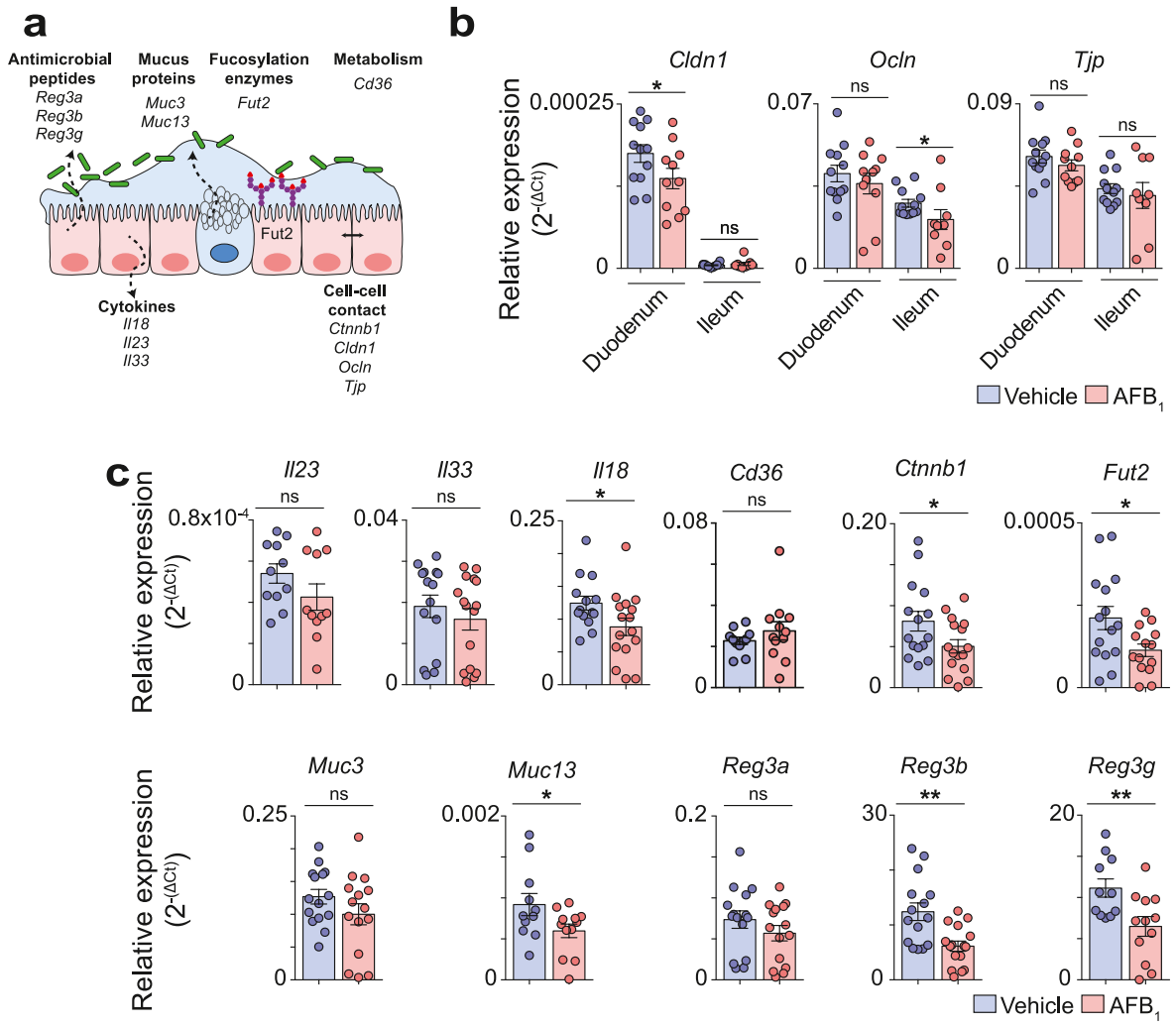

Fig. 4. Maternal AFB_1 exposed offspring display intestinal downregulation of microbial-related gene expression. a. Schematic representation of epithelial-expressed genes relevant for intestinal mucosal. Mother females were treated with AFB_1 400 μg/kg body weight. **b.** Relative expression of indicated genes were determined by RT-PCR in intestinal epithelial cells purified by flow cytometry cell sorting from duodenum and ileum intestinal portions. Specific transcript levels were determined relative to *Gapdh* and *Epcam*. Vehicle or AFB_1 n = 9 to 12. **c.** Relative expression of indicated genes were determined by RT-PCR in jejunal whole tissue. Specific transcript levels were determined relative to *Gapdh* and *Actb* mRNA levels. Bars depict mean, each symbol is an individual mouse, error bars represent ± s.e.m. Vehicle n = 11; AFB_1 n = 12; n represents independent animals. Data represents 2 independent experiments. For statistical analysis, a two-tailed Mann–Whitney *U* test was used. *P < 0.05; **P < 0.01; ns, not significant. *Cldn1*: Claudin 1; *Ocln*: Occludin; *Tjp*: Tight Junction Protein 1 or Zonula occludens-1; *Cd36*: CD36 molecule; *Ctnnb1*: Catenin beta 1; *Fut2*: Fucosyltransferase 2; *Il*: interleukin; *Muc*: mucin; *Reg*: Regenerating Family Member.

2.5. Leukocyte populations

During flow cytometry analysis, cell populations were defined as: B cells were defined as: $CD19^+MHCII^+$; T cells: $TCR\alpha\beta^+$ or $TCR\gamma\delta^+$ cells; DC cells: $CD11c^+MHCII^+$; Macrophages: $CD64^+CD11b^+$; ILC2: $CD45^+Lin^-Thy1.2^+KLRG1^+Sca\text{-}1^+CD25^+$; ILC3: $CD45^+Lin^-Thy1.2^{high}$. The lineage (Lin) cocktail included CD3ϵ, CD8α, CD19, CD11c, CD11b, Ter119, Gr1, TCRβ, TCRγδ and NK1.1. Flow cytometry gate strategy to define leukocyte populations is displayed in Figs. 1 and 3.

2.6. Antibody list

Cell suspensions were stained with (all antibodies were purchased from Biolegend unless otherwise indicated): anti-CD45 (30-F11, FITC); anti-CD45 (30-F11, AF-700, Invitrogen); anti-CD11c (N418); anti-CD11b (Mi/70); anti-CD8α (53-6.7); anti-CD19 (6D5); anti-NK1.1 (PK136); anti-CD3ϵ (145-2C11); anti-TER119 (TER-119); anti-Gr1 (RB6-8C5); anti-CD4 (RM4-5, GK1.5); anti-CD25 (PC61.5, Invitrogen); anti-CD90.2 (53-2.1); anti-TCRβ (H57-597); anti-TCRγδ (GL3); anti-KLRG1 (2F1/KLRG1); anti-Ly-6A/E (Sca1, D7); anti-IL-17 (TC11-18H10.1); anti-IL-22 (1H8PWSR, eBioscience); anti-IL-5 (TRFK5, BD Biosciences); antil-IL-13 (eBio13A, Invitrogen); anti-Amphiregulin (polyclonal, R&D) anti-CD64 (X54-5/7.1); anti-MHCII (M5/114.15.2, eBioscience); anti-CD31 (390); anti-EpCam (G8.8); anti-GMCSF (MP1-22E9); anti-IFNg (XMG1.2, Biolegend); anti-streptavidin fluorochrome conjugates from Biolegend; anti-CD16/CD32 (93, BD Pharmingen). LIVE/DEAD Fixable Aqua Dead Cell Stain Kit was purchased from Invitrogen.

2.7. Quantitative reverse transcription PCR

RNA from sorted epithelial cells was extracted using RNeasy micro kit (Qiagen) according to the manufacturer's protocol. Small intestine was collected and snap-frozen in liquid nitrogen. Tissues were lysed using RLT Plus Lysis and buffer Reagent Dx (0,5% v/v). Stainless spheres with 3 mm diameter were used in the TissueLyser II (30 Hz for 2 min). Lysates were centrifuged at full speed 3 min at 4 °C. Supernatants were collected to isolate RNA. RNA concentration was determined using the

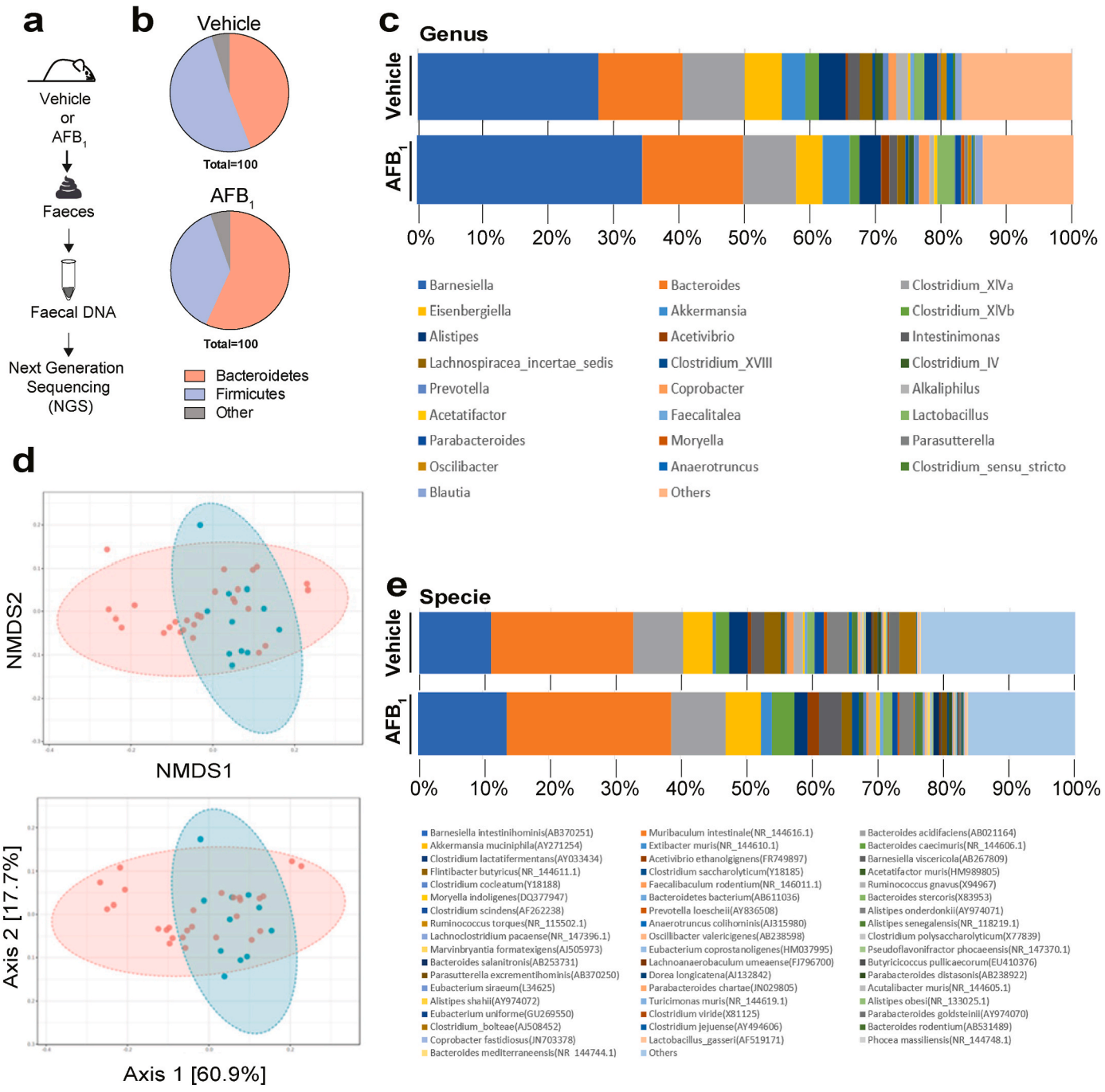

Fig. 5. AFB_1 maternal exposure leads to alterations of intestinal microbial communities in offspring. a. Schematic representation of faecal DNA analysis by NGS to quantify relative abundance of predominant bacteria in gut. Mother females were treated with AFB_1 400 μg/kg body weight. **b-d.** NGS evaluation of the microbial diversity of faecal content from maternal AFB_1- or vehicle-exposed offspring. **b.** Bacterial phylum. **c.** Genus biodiversity. **d.** β-diversity analysis. Non-metric Multi-dimensional Scaling-NMDS and Bray-Curtis dissimilarity index. Calculated from one independent experiment. **e.** Species biodiversity. Data is representative of 2 independent experiments. Vehicle, n = 12; AFB_1, n = 19.

Nanodrop Spectrophotometer (Nanodrop Technologies). For TaqMan assays (Applied Biosystems) RNA was retro-transcribed using a High-Capacity RNA-to-cDNA Kit (Applied Biosystems), followed by a pre-amplification PCR using TaqMan PreAmp Master Mix (Applied Biosystems). TaqMan Gene Expression Master Mix (Applied Biosystems) was used in quantitative reverse transcription PCR (qRT-PCR). qRT-PCR analysis was performed using StepOne and QuantStudio 5 Real-Time PCR systems (Applied Biosystems). mRNA levels of *Gapdh*, *Actb* and/or *Epcam* genes were used to normalize quantification of specific transcript levels of genes of interest. When multiple endogenous controls were used, these were treated as a single population and the reference value calculated by arithmetic mean of their CT values. The mRNA analysis was performed as previously described (Van De Pavert et al., 2014). In brief, we used the comparative CT method ($2^{-\Delta CT}$), in which ΔCT(gene of interest) = CT(gene of interest) − CT(housekeeping reference value).

2.8. TaqMan gene expression assays

TaqMan Gene Expression Assays (Applied Biosystems) were the

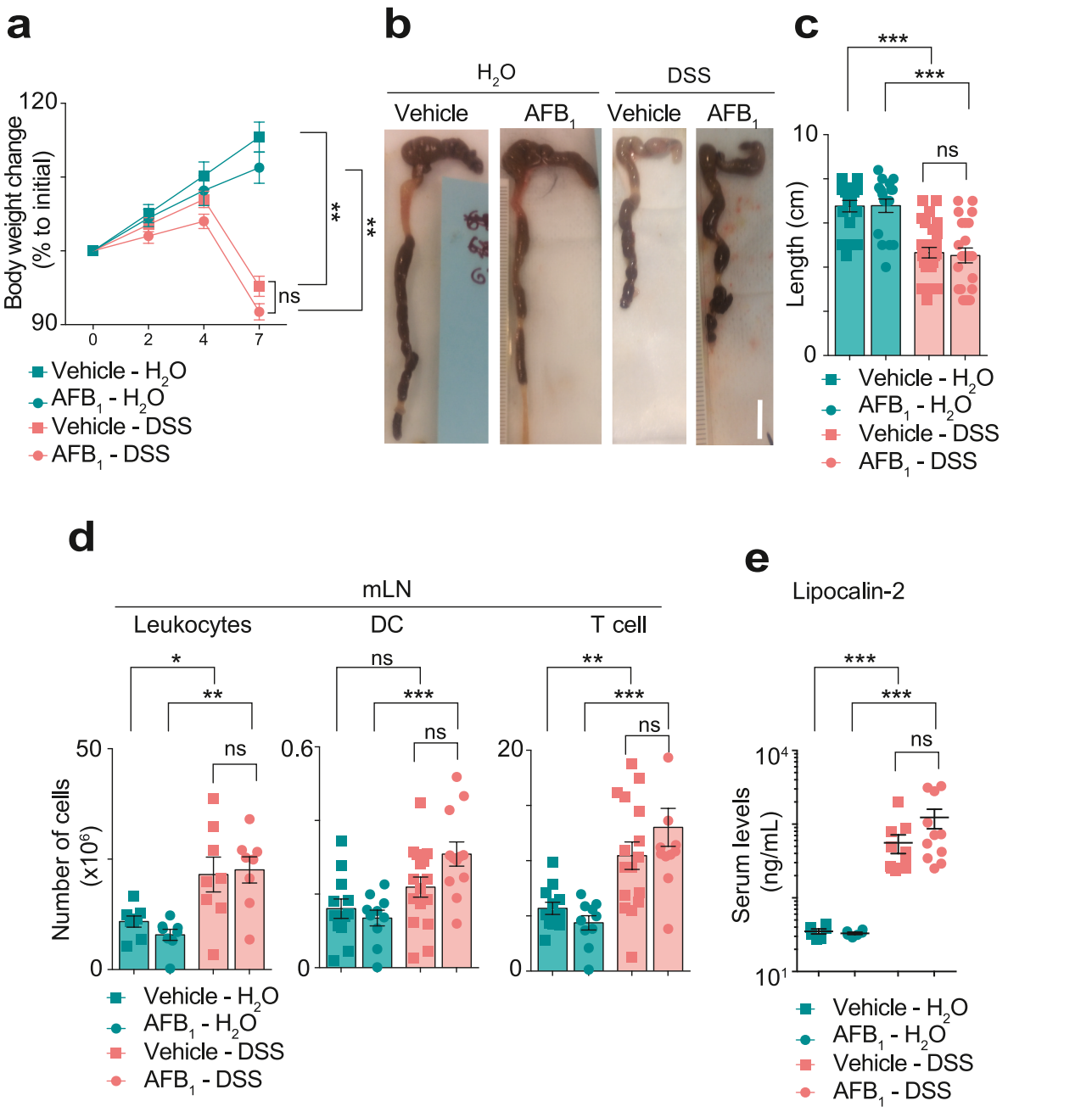

Fig. 6. AFB_1 maternal exposed offspring display unaltered susceptibility to inflammatory stimuli. Mice 3- to 4-week-old born to vehicle- or AFB_1-exposed females were treated with dextran sodium sulphate (DSS) or water for seven days. Before DSS treatment, mother females were treated with AFB_1 400 μg/kg body weight. **a.** Body weight changes were determined over the course of DSS treatment. **b.** Representative photographs of colon. Bar represent 1 cm. **c.** Colon length of distal colon at seven days post-DSS treatment. Vehicle (H_2O), n = 19; Vehicle (DSS), n = 28; AFB_1 (H_2O), n = 18; AFB_1 (DSS), n = 22. **d.** Absolute number of mesenteric lymph node (mLN) leukocytes by flow cytometry analysis. Vehicle (H_2O), n = 8 to 10; Vehicle (DSS), n = 8 to 16; AFB_1 (H_2O), n = 8 to 10; AFB_1 (DSS), n = 8 to 12. **e.** Lipocalin-2 levels quantified by ELISA in offspring serum. Vehicle (H_2O), n = 6; Vehicle (DSS), n = 11; AFB_1 (H_2O), n = 6; AFB_1 (DSS), n = 11. All data are combined from 3 independent experiments. n represents individual animals. Bars depict mean, each symbol is an individual mouse, error bars represent ± s.e.m. For statistical analysis, a two-tailed Mann–Whitney *U* test was used. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant; AFB_1-aflatoxin B_1.

following: *Gapdh* Mm99999915_g1; *Actb* Mm02619580_g1; *Epcam* Mm00493214_m1; *Reg3a* Mm01181787_m1; *Reg3b* Mm00440616_g1; *Reg3g* Mm00441127_m1; *Muc3* Mm01207064_m1; *Muc13* Mm00495397_m1; *Cd36* Mm01307193_g1; *Fut2* Mm01205565_m1; *Il18* Mm00434225_m1; *Il23* Mm00518984_m1; *Il33* Mm00505403_m1; *Ctnnb1* Mm00483039_m1; *Cldn1* Mm00514374_m1; Ocln Mm00500912_m1; *Tjp* Mm00493699_m1.

2.9. Dextran sodium sulphate-induced colitis

Dextran Sodium Sulphate (DSS; molecular mass 36′000–50′000 Da; MP Biomedicals) was added into drinking water 3% (w/v) for 7 days. Body weight was assessed daily. At days 8 and 9 animals were sacrificed, the length of the colon was determined and serum, intestine and mesenteric lymph node were collected.

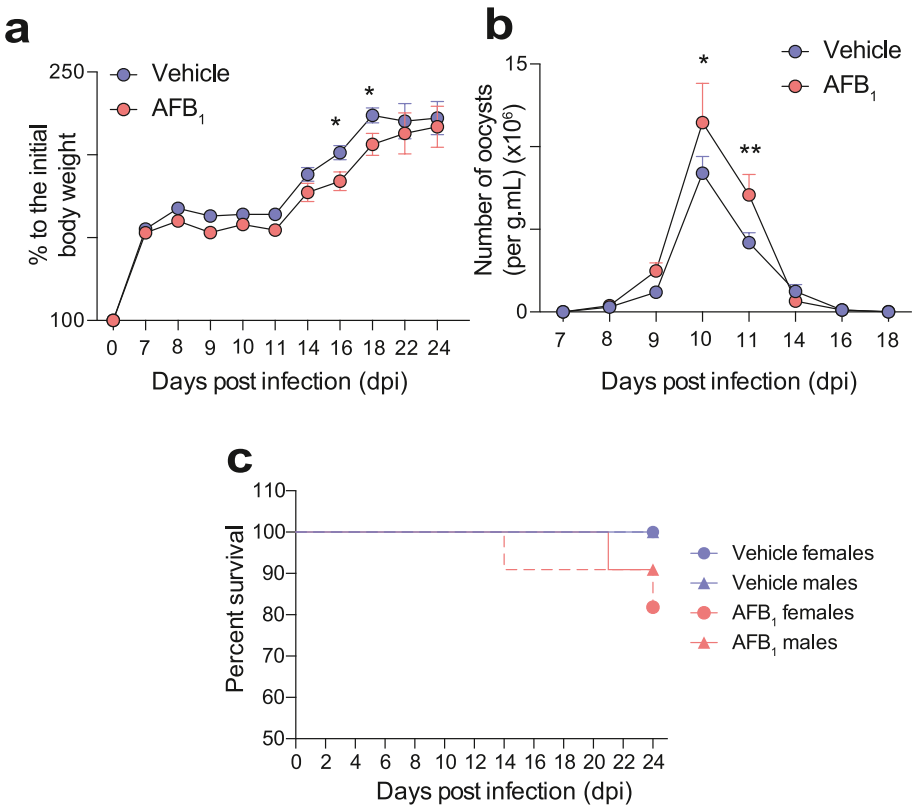

Fig. 7. Maternal AFB_1 exposure leads to offspring more susceptible to intestinal infection. 2.5-week-old offspring born to vehicle- or AFB_1-exposed females were orally infected with 1000 *E. vermiformis* sporulated oocysts. Before *E. vermiformis* infection, mother females were treated with AFB_1 400 μg/kg body weight. **a.** Body weight changes over the course of infection until 24 days post infection (dpi). Vehicle, n = 11 to 44; AFB_1, n = 18–43 **b.** Number of oocysts collected from individual mouse faeces between 7 and 18 dpi. Vehicle, n = 11 to 43; AFB_1, n = 21 to 42. **c.** Survival of maternal AFB_1- or vehicle-exposed offspring over the infection period. Vehicle, n = 44; AFB_1, n = 43.n represents independent animals. Data represents summary of 2 independent experiments, which included 6 independent litters of vehicle or AFB_1. For statistical analysis, a two-way ANOVA multiple comparisons test was used. *P < 0.05; **P < 0.01; not significant, when not indicated.

2.10. *Eimeria vermiformis* infection

Animals were infected with *E. vermiformis* as previously described in detail (Figueiredo-Campos et al., 2018). Briefly, oocysts were washed three times with deionized water, floated in sodium hypochlorite and counted using a Fuchs–Rosenthal chamber. Mice received 500 oocysts of *E. vermiformis* by oral gavage in 100 μl of water. To determine burden of infection, animals were individually isolated in beakers for 3–5 min and faeces (two to three pellets) were collected. Faeces collection and body weight monitorization was performed every 2 or 3 days between 7- and 18-days post infection.

2.11. *Microbiota assessment by next generation sequencing (NGS)*

Abundance and relative importance of main gut bacterial taxa were assessed by NGS using an Illumina Miseq System. Briefly, thirty-nine faecal samples from maternal AFB_1- and thirty vehicle-exposed pups (15-days-old) were diluted in sterile PBS. Bacterial DNA was extracted (E.Z.N.A.® Stool DNA Kit, Omega BIO-TEK) and further quantified by fluorometry. The V3 and V4 regions of the 16S rRNA gene were amplified with primers containing Illumina adapter overhang nucleotide sequences, purified and further reamplified in a limited-cycle PCR reaction to add dual indexes (i7 and i5) (Nextera XT Index Kit, Illumina, San Diego, CA). After clean up, the final library was validated and the amplicons were quantified, normalized and pooled as described elsewhere(Caldeira et al., 2021). After sequencing, the denaturated libraries fragments were de-multiplexed, quality-filtered and operational taxonomic units (OTUs) determined by clustering reads to 16S reference databases (ex: Greengenes and ARB Silva). Indicspecies package allowed the identification of differences in gut microbiota patterns of both maternal AFB_1- and vehicle-exposed animals at the different taxonomic ranks. Relative abundance of different bacterial taxa was evaluated between maternal AFB_1 exposed and unexposed litters. Alfa-diversity (α-diversity) index was measured using the Shannon diversity index. Beta-diversity (β-diversity) between maternal AFB_1 exposed and unexposed samples was evaluated using the Bray-Curtis dissimilarity index.

2.12. *ELISA*

For lipocalin-2 quantification in serum, blood was collected by vein puncture into Microtainer® blood collection tubes (BD) and serum was obtained by centrifugation at 2000*g* for 10 min. Relative serum lipocalin levels were quantified using LEGEND MAX™ Mouse NGAL (Lipocalin-2) ELISA Kit (Biolegend) using manufacter's instructions.

2.13. *Statistics*

Results are shown as mean ± s.e.m. Statistical analysis was performed using GraphPad Prism software (version 6.01). Comparisons between two samples were performed using Mann–Whitney *U* test. For multiple comparisons a two-way ANOVA multiple comparisons test was used. Results were considered significant at *P < 0.05, **P < 0.01, ***P < 0.001.

3. Results

3.1. *Intestinal secondary lymphoid organs of maternal AFB_1-exposed offspring*

To investigate the impact of maternal AFB_1 exposure on the intestinal immune system of offspring, pregnant females received AFB_1 (200 or 400 μg/kg body weight of the animal) in gelatine pellets 3 times a week from the E11.5 until the weaning of the progeny (during their pregnancy and breastfeeding). Signs of maternal toxicity were not observed after treatment with AFB_1. Size litter and pup weight were similar in both AFB1 and control groups (Fig. Suppl. 1a-c); and no gross observable malformations were detected. This indicates no major disturbance of overall development *in utero*, which is in accordance with the fact that

the most sensitive period of embryonic development to AFB_1 occurs until embryonic day 8.5 (E8.5) (Arora et al., 1981).

The intestinal immune system comprises secondary lymphoid organs (SLOs), namely mesenteric lymph nodes (mLN) and Peyer's patches (PP), that allow resident lymphocytes to encounter antigen-presenting cells promoting tissue immune-surveillance and rapid immune responses. Critical hematopoietic progenitors emerging in foetal liver initiate the morphogenesis of SLOs between embryonic day 9.5 (E9.5) and 16.5 (E16.5) completing their development as mature lymphoid structures during postnatal period (Coles and Veiga-Fernandes, 2013).

To investigate the impact of maternal AFB_1 exposure in the development of gut-draining SLO in the offspring, we analysed PP and mLN. Macroscopic alterations of mLN were not detected and number of PP was similar in vehicle- and AFB_1-treated offspring (Fig. 1f). Accordingly, flow cytometry analysis of major leukocyte populations (Fig. Suppl. 1c) revealed comparable frequency and absolute number of innate and adaptive immune cells in mLN and PP of both groups (Fig. 1c and d; g-h). These results indicate that under these particular conditions of exposure, AFB_1 did not disturb the formation of intestinal SLO neither their main leukocyte populations.

3.2. Maternal AFB_1 exposure leads to expansion of lymphocyte number in offspring gut

In addition to organized lymphoid tissues, intestinal mucosal comprises a large population of scattered immune cells in the gut epithelium, known as the intraepithelial lymphocytes - IELs, as well as mingled in connective tissue, lamina propria (LP), that survey gut compartment and respond to environmental cues (Hoytema van Konijnenburg et al., 2017; Thompson et al., 2019).

Flow cytometry analysis showed that, while frequency of major adaptive and innate immune cell subsets was mostly unchanged (Fig. 2b, e), an overall increase of leukocyte number of IEL and LP compartments of maternal AFB_1-exposed progeny was observed (Fig. 2c, f), translating into an incrementation of lymphocyte B, TCRγδ and TCRαβ, either CD4 or CD8α, T cell number (Fig. 2c, f). Thus, these results indicate that adaptive immune cell populations are expanded in maternal AFB_1-exposed progeny's intestine.

3.3. Effect of maternal AFB_1 exposure on ILC2 innate cytokine production

Innate lymphoid cells (ILCs) constitute the innate counterpart of T lymphocytes and are highly enriched in mucosal tissues, including the gut. To investigate the effect of maternal AFB1 ingestion, we analysed by flow cytometry major ILC subsets in gut taking advantage of specific cell-surface protein and transcription factor expression to identify each population (Fig. 3a). Despite similar total number, the frequency of ILC amongst leukocytes was slightly reduced in maternal AFB_1-exposed gut (Fig. 3b and c). On the other hand, analysis of individual ILC subsets, showed that the frequency of ILC2 or ILC3 within the ILCs were similar (Fig. 3b and c) and that ILC3-specific cytokine subsets was generally unperturbed (Fig. 3d–f). Yet, revealed a strong reduction of cytokine expression, namely interleukin (IL)-5, IL-13 and amphiregulin (AREG), by maternal AFB_1-exposed ILC2s as compared with vehicle-exposed group (Fig. 3g–j). These data indicate that ILC2s present in maternal AFB_1-exposed gut are deficient in cytokine production.

3.4. Impact of maternal AFB_1 exposure in epithelial gene expression

AFB_1 toxicity has been associated with alterations of intestinal epithelial cells (Akbari et al., 2017). This study sought to investigate whether maternal AFB_1 exposure disturbs intestinal epithelium in offspring by quantifying the expression of epithelial-expressed genes critical for intestinal mucosa homeostasis (Fig. 4a). While mRNA transcript levels of proinflammatory cytokines *Il23* and *Il33*, as well as *Cd36*, a key gene involved in lipid metabolism, were unaffected (Fig. 4c), the expression of genes involved in innate immune response against microbial insults, such as *Il18*, *Muc13*, *Reg3b* and *Reg3g*, were approximately 2-fold decreased in maternal AFB_1-exposed offspring gut.

Additionally, analysis of *Fut2* transcript levels, encoding for the enzyme fucosyltransferase key in the transference of sugar molecules to cell surface and critical in host–commensal symbiosis (Omata et al., 2018; Pickard et al., 2014), revealed a 2-fold downregulation in maternal AFB_1-exposed intestines when compared with control guts (Fig. 4c). Interestingly, *Ctnnb1*, encoding β-catenin, a component of adherent junctions in epithelia mediating cell-cell adhesion, was also downregulated (Fig. 4b). Some of tight junctional membrane proteins were also found downregulated in maternal AFB_1-exposed guts (Fig. 4b). Together these results indicate that expression of critical epithelial components involved in epithelial integrity, innate immune defence and crosstalk with gut microbial communities are changed in offspring upon maternal AFB_1 exposure.

3.5. Maternal AFB_1-exposed offspring display changes in intestinal microbiota

To investigate whether maternal exposure to AFB_1 led to alterations of gut microbiota in their offspring, we employed next generation sequencing (NGS) (Fig. 5a). Comparison of phylum biodiversity between exposed and unexposed litters revealed an increase of Bacteroidetes and a decrease of Firmicutes (Fig. 5b).The Firmicutes/Bacteroidetes ratio: was 0.81 in unexposed litters, lowering to 0,58 in AFB1-exposed (p-value <0,05; confidence level 95%).

A deeper analysis identified an increase in relative abundance of *Barnesiella* genus and increase of some *Bacteroides* species known to colonize the young intestine (Fig. 5c). α-diversity evaluated by Shannon index showed no statistical differences between groups, proving that microbiota diversity was unaffected in exposed litters. Still, AFB_1 maternal exposure may have induced changes in bacterial composition, as β-diversity between AFB_1 and control groups were statistically significant (p-value of Bray-Curtis dissimilarity index <0.041) (Fig. 5d). Several bacterial species were significantly reduced, while others increased in comparison to vehicle-exposed mice (Fig. 5e). .

3.6. Susceptibility to chemical-induced colitis is unperturbed by maternal AFB_1 exposure

Alterations in gut immune cell populations, as well as disturbance of the epithelia-derived gene expression and gut microbiota, have been implicated in intestinal inflammation and susceptibility to a variety of infections (Elinav et al., 2011; Ma et al., 2019; Ngo et al., 2022; Panda and Colonna, 2019). To investigate whether these intestinal alterations observed in maternal AFB_1-exposed offspring were associated with changes in immune response to external assaults, we tested their susceptibility to enteric aggressions.

We firstly took advantage to a well-established chemical-induced colitis model using dextran sodium sulphate (DSS). This is widely used to evaluate susceptibility to inflammatory stimuli (Boismenu et al., 1999). Analysis of all DSS-treated groups showed drastic weight loss, colon shortening, leukocyte infiltration in mLN and increased of lipocalin serum levels in comparison with water treated mice (Fig. 6a–e), which are indicative of tissue damage and induction of intestinal inflammatory response. Comparison of maternal AFB_1-exposed versus vehicle-exposed offspring inside the DSS-treated group revealed no differences, indicating that maternal AFB_1 intake did not worsen the susceptibility of offspring to DSS-induced colitis.

3.7. Maternal AFB_1-exposed progeny is more susceptible to intestinal parasite infection

Attending to downregulation of genes involved in microbial defence

(Fig. 4), we sought to investigate whether maternal AFB_1 exposure-induced intestinal changes that increased the susceptibility to intestinal infections. For that we used the intracellular protozoan *Eimeria vermiformis* that infects murine enterocytes and that has been used to study mucosal immunity (C. Ferreira et al., 2020; Figueiredo-Campos et al., 2018). We examined maternal AFB_1- and vehicle-exposed offspring infected with *E. vermiformis* at 2-week-old (Fig. 7a). During this early-life period, mice increase their weight overtime. Interestingly, maternal AFB_1-exposed offspring showed lower body weight gain over the time of the infection when compared with vehicle-exposed progeny (Fig. 7a). Accordingly, quantification of oocysts shed into the faeces displayed higher oocysts burden (Fig. 7b), which was associated with some mortality amongst AFB_1-exposed progeny group (dead pups: AFB_1, 3 out of 43; vehicle, 0 out 44), when compared with vehicle-exposed (Fig. 7c). These results indicate that maternal AFB_1 exposure leads to a higher susceptibility to intestinal infection in offspring.

4. Discussion

Development of intestinal barrier initiates *in utero* and continues during the perinatal period (Torow et al., 2016). This process involves not only the formation of the epithelial monolayer, but also the establishment of resident immune cell populations, the development of lymphoid organs and concurrently the colonization by microbial communities at the mucosal body surface. The intestinal barrier maturation, despite following a developmental program, is dependent on environmental cues. We recently showed that lymphoid organ morphogenesis are markedly determined by the uptake of dietary retinoic acid during gestation and the perinatal period (Goverse et al., 2016; Van De Pavert et al., 2014), suggesting that exposure to contaminant compounds present in foods, as mycotoxins, may potentially lead to intestinal immune developmental alterations and increase susceptibility to disease (Godfrey et al., 2010).

Here, we show that, while gut-associated lymphoid organ (mLN and PP) development and its leukocyte subsets were not changed in progeny of female mothers fed with AFB_1-containing diet during gestation and lactation, this maternal exposure led to expansion of immune cell populations in LP and IELs and reduction of cytokine production by ILC2. Furthermore, these immune changes were associated with alterations in epithelial gene expression and gut microbiota composition. Interestingly, despite susceptibility to inflammatory stimuli was not worsen, maternal AFB_1-exposed offspring were more prone to infection when in contact with intestinal pathogenic intracellular protozoan.

After ingestion, AFB_1 is quickly absorbed from the gastrointestinal tract to blood stream being metabolized to diverse toxic compounds reaching internal organs, including the mammary gland and placenta, which are transferred to the progeny (Hernández and E, 1996; L. Wang et al., 2022). AFB_1 metabolites can be detect in the progeny tissues, including in the liver (Allameh et al., 1989; Chawanthayatham et al., 2015).

Mounting evidence show that AFB_1 exposure during embryonic and perinatal period negatively affects the immune system (Ivanovics et al., 2021; Qureshi et al., 1998). However, most of these studies present limitations to understand the influence of maternal dietary cues in the progeny through gestation and lactation processes, as chick or zebrafish were used as models (Dietert et al., 1983; Ivanovics et al., 2021; Potchinsky and Bloom, 1993; Qureshi et al., 1998). Some studies used weanling mammals to address the impact of AFB_1 early exposure in immune system of developing animals (Harvey et al., 1988; Panangala et al., 1986; Raisuddin et al., 1990; Reddy et al., 1987). However, this approach does not assess how mother's AFB_1-containing diet influence the developing progeny during embryogenesis and breastfeeding. More recently, some studies showed that mammal offspring from females under AFB_1 dietary exposure display thymic alterations and impaired circulating lymphocytes function (Mocchegiani et al., 1998; Silvotti et al., 1997). Nevertheless, the impact of maternal AFB_1 exposure in intestinal barrier homeostasis and function of mammal progeny was not explored.

In this study, analysis of the enteric immune system, epithelial cells, microbiota and intestinal function was performed in offspring of murine female mothers that continuously ingested AFB_1 during pregnancy and breastfeeding. Murine females received 9.2–12 μg of AFB_1 per animal per day (3 times a week), in a gelatine pellet, which is equivalent to a dosage 0.123–0.160 μg/kg d^{-1} in human. This is close to the average daily intake range of AFB_1 by the human population in the high-risk areas (184.1 μg/kg d^{-1}, taking into account the consumption of foodstuffs commonly contaminated with AFB_1, such as cereals and oilseeds, especially in developing countries (Filazi and Sireli, 2013; Kamika and Takoy, 2011; Williams et al., 2004). Based in Williams et al. (2004), the human exposure dose varies between countries, largely as a function of diet. For example, in Mozambique human population is exposed to 38.6–183.7 ng · kg−1 d−1; in Swaziland to 11.4–158.6; and in southern Guangxi province of China people are exposed to 11.7–2027 ng · kg−1 d−1. Yet, the exposure in the United States is 2.7 ng · kg−1 d−1.

Thus, this study embodies a realistic dose and route of administration to assess the developmental effects in intestinal barrier of progeny induced by food borne toxicants present in maternal dietary, in this particular case, AFB_1, through the *utero* and during lactation.

Critical foetal hematopoietic progenitors emerge in foetal liver and start to colonize the SLO anlagen structures by embryonic day 9.5 (E9.5) completing their development as mature LN or PP during the postnatal period (Coles and Veiga-Fernandes, 2013). AFB_1 is a potent hepatocarcinogen (Deng et al., 2018; Wogan, 1973), suggesting that foetal liver hematopoietic precursors, and consequently SLO formation, could be affected by prenatal and post-natal maternal AFB_1 exposure. In fact, DNA adducts were detected in embryonic liver upon maternal AFB_1 exposure gavage (Chawanthayatham et al., 2015). However, its hepato-physiological negative impact is restricted to the developmental period before E9.5. In this study, maternal AFB_1 exposure started at E11.5, which is coherent with the fact that intestinal SLO, mLN and PP, were not affected. Interestingly, weights of mLN and tracheal LN of weanlings rats were not affected significantly by direct AFB_1 administration (Raisuddin et al., 1990).

On the other hand, our study shows a substantial increase of the absolute number of immune cells in intestinal LP and IEL. Most of the studies showed that AFB_1 immunosuppressive properties are associated with reduced lymphocytes populations (Raisuddin et al., 1990). Despite the absolute number of lymphocytes is not presented, a study reported a decrease of T cell frequency in intestine upon direct administration of AFB_1 to chicken (He et al., 2014). Absolute cell number of lymphocytes depends on the size of gut. Interestingly, an increase in the small intestine length upon AFB_1 treatment is observed (Yunus et al., 2011).

Studies showed that immunosuppressive properties of AFB_1 are associated with reduced lymphocytes populations (He et al., 2014; Raisuddin et al., 1990). Here, we observed a substantial increase of the immune cell absolute number in intestinal LP and IEL; which is in line with previous investigations showing that prolonged exposure to AFB_1, not acute, resulted in the increase of splenic T cells and hyperplasia of the bone marrow in rats (Qian et al., 2014). In addition, it was recently shown an increased number of leukocytes in abdomen of zebrafish embryos under AFB_1 exposure (Ivanovics et al., 2021). Observations of increased humoral immune response during initial stages of exposure to AFB_1 later followed by a decrease in humoral immune response have been reported (Yunus et al., 2011). In the same study, while short term AFB_1 exposure resulted in decreased CD8 T cells and in an immunosuppressive profile, a prolonged exposure resulted in increased number of T cells and pro-inflammatory immune response (Qian et al., 2014). Thus, indicating that differences amongst publications can potentially be due to different experimental designs, exposure dose and to the different animal models used, as well as to analysis timings.

On the other hand, the AFB_1 metabolization, which is dependent on dose, species, and age, must be considered. A foetal form of CYP3a4 has

been observed in foetal liver within 2 months of conception (Lacroix et al., 1997) indicating that the foetus may metabolically generate reactive epoxides following transplacental transfer of maternally ingested AFB_1 (Partanen et al., 2009). Foetal livers catalyse the formation of the epoxide at similar rates to adults, but have a lower capacity to protect against toxicity (Doi et al., 2002).

Immune cell populations in maternal AFB_1-exposed guts were accompanied by significant decrease of expression of epithelial-related genes encoding components of tight and adherent junctions, such as *Cldn1* and *Ocln*, critical for intestinal barrier integrity (Ngo et al., 2022). These findings are in accordance with previous reports showing epithelial disruption in chicken (Yin et al., 2016; Yunus et al., 2011; Zhang et al., 2014) and changes in the expression of ZO-1, claudins and occludin due to exposure to AFB_1 (Caloni et al., 2006; X. Chen et al., 2016; Gao et al., 2017; Wu et al., 2019). Additionally, immune mediators, critical roles in mucosa barrier protection, such as *Muc13*, *Reg3b*, *Reg3g* and the *Il18*, involved in type 1 immune responses, were also reduced in AFB_1-exposed progeny (Muñoz et al., 2015; Okamura et al., 1995). Interestingly, active AFB_1 metabolites in milk were shown to interact with mucin protein secretions *in vitro* (Caloni et al., 2006; Wu et al., 2019), which is in line with the downregulation of mucins in maternal AFB_1-exposed guts in this study.

The development of a stable gut microbiota is intimately associated with early development of host immunity, in such way, that disturbances have been linked to several diseases in infancy and adulthood. Abnormal changes in gut microbiota (dysbiosis) were observed in mice upon maternal AFB_1 exposure, such as reduction in SCFA producers. Interestingly, this was associated with a reduction of *Fut2* transcript levels, a fucosylation enzyme that is critical in host–commensal symbiosis (Pickard et al., 2014).

Previous studies have implicated AFB_1 in intestinal microbiota changes (Ishikawa et al., 2017; Liew and Mohd-Redzwan, 2018; J. Wang et al., 2016; Xiai Yang, Liangliang Liu, 2017). Accordingly to described previously, we observed an increase in Bacteroidetes frequency at the expense of Firmicutes phyla upon maternal AFB1 exposure (Ishikawa et al., 2017; Xiai Yang, Liangliang Liu, 2017*)*. The lowering of Firmicutes/Bacteroidetes ratio has been recognized to lead to dysbiosis and has been related to several inflammatory bowel diseases (Frank et al., 2007; Nishida et al., 2018). Disruptive effects of AFB_1 were reported as dose dependent (J. Wang et al., 2016) and to disrupt a number of gut-microbiota dependent metabolic pathways (Zhou et al., 2019, 2021), thus supporting our observations of intestinal microbiota modification upon maternal AFB_1 exposure in early-life. Among bacterial species diminished in AFB_1 exposed progeny were several species proven beneficial, especially members of the Firmicutes phylum known to be important butyrate producers, which enhances epithelial barrier integrity and inhibits inflammation (Geirnaert et al., 2017). Moreover, an increase in sulphate-reducing bacteria was observed in several exposed samples (n = 19) (especially *Desulfovibrio piger* and *Bilophila wadsworthia),* which are associated with gut inflammation (Devkota et al., 2012). Some species were decreased, particularly *Flintibacter butyricus*, known for its importance in the production of Short-Chain Fatty Acids (SCFA) or *Bacteroides acidifaciens* related to the gut immune system maturation and to prevent metabolic diseases (Singh, 2019)"

Maternal AFB_1-exposed ILC2 presented strong reduction of IL-5, IL-13 and AREG levels. (Boismenu et al., 1999; O'Connor et al., 2009; H. Park et al., 2005). Extensive literature linked ILCs to intestinal inflammatory disorders (Panda and Colonna, 2019) and disruption of T cell populations can determine intestinal inflammatory responses (Boismenu et al., 1999; O'Connor et al., 2009; H. Park et al., 2005). Also intestinal microbial community alterations have been associated with gastrointestinal inflammation (Ngo et al., 2022; Nishida et al., 2018). The observed AFB_1-induced intestinal immune alterations could, thus, point towards an increased inflammatory response. However, maternal AFB_1-exposed mice displayed similar susceptibility to DSS-induced inflammatory stimuli when compared to the AFB_1-free controls.

Some lymphocyte populations and anti-inflammatory cytokines can counteract inflammation. While expansion of IL-13-producing ILC2s has been shown to play a detrimental role in colitis (Camelo et al., 2012), AREG-producing ILC2s has been shown to contribute to tissue repair during DSS-induced colitis (Monticelli et al., 2015). Thus, ILC2s may either promote or block intestinal pathogenesis, depending on the disease setting. On the other side, increased levels of the anti-inflammatory cytokine IL-10 upon AFB_1 exposure has been reported (Marin et al., 2002; G. M. Meissonnier et al., 2008; Qian et al., 2014). Whether immune cell populations in AFB_1-exposed guts are under an immunosuppressive cytokine environment requires further investigation.

Interestingly, maternal AFB_1 dietary exposure led to a higher susceptibility to intestinal infection by *E. vermiformis*, an intracellular protozoan closely related to human infectious pathogens, such as *Cryptosporidium* spp, that infect intestinal epithelial cells (Shields and Olson, 2003). These findings support previous works showing that AFB_1 is immunosuppressive, as AFB_1 has been shown to increase susceptibility to infections (Cusumano et al., 1990; Joens et al., 1981; Venturini et al., 1996). Microbiota direct a protective immune response against parasitic infections (Benson et al., 2009; Lu et al., 2021; Stange et al., 2012; Tierney et al., 2004) and ILC2 are necessary to prevent protozoan pathogen infection (Jones et al., 2010). Expression of cytokines, such as IL-18 (Muñoz et al., 2015) and IL-13 (McKenzie et al., 1998), are also dynamically involved in parasitic infections (Hong et al., 2006). In addition, increased levels of defensins RegIIIβ and RegIIIγ have been implicated in higher parasite *E. falciformis* burden (Stange et al., 2012). On the other side, $CD4^+$ T cells play the role of effector cells against primary infection of *E. vermiformis*, while γδ T cells play the role of regulatory cells (Roberts et al., 1996). Thus, maternal AFB_1-induced alterations of these immune players in progeny are coherently associated with suppression of intestinal immune defence against pathogens.

Whether the observed changes in intestine of the progeny are direct or secondary effects of AFB_1 remains unknown. Recent studies reported that maternal gut bacteria can drive intestinal inflammation in offspring (De Agüero et al., 2016) and that matern al factors are transferred to progeny (E. Kim et al., 2022; S. Kim et al., 2017).

5. Conclusion

The present study shows alterations of the intestinal immune cell populations, as well as reduction of the epithelial gene expression and microbiota modification linked to the decay of intestinal immune response to parasitic infections in young individuals upon AFB_1 exposure. These results support previous literature acknowledging AFB_1 as an immunosuppressive agent and further shows that dietary maternal AFB_1 negatively impacts in intestinal immune mucosal barrier and function.

AFB_1-inducied intestinal immunological alterations may likely increase susceptibility to infections and potentially contribute to failure of vaccination programs in developing countries, where fungal growth and mycotoxin formation is favourable (Githang'a et al., 2019; Karlsson et al., 2022; Tesfamariam et al., 2022). Additional investigation is required to unveil the underlying mechanisms and to further understand long-lasting impact of maternal AFB_1 exposure.

Funding

This research was funded by Fundação para a Ciência e a Tecnologia (FCT) through national funds EarlyMyco (PTDC/MED-TOX/28762/2017), *investigator* FCT Exploratory (IF/00425/2015/CP1324/CT0001) and CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020).

Institutional review board statement

All animal experiments were conducted in accordance with the guidelines of the Animal Care and Use approved by national and local Animal Welfare Body (AWB), respectively, Direção Geral de Veterinária and FC ethical committees.

CRediT authorship contribution statement

Patricia Bastos-Amador: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Elsa Leclerc Duarte:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Funding acquisition. **Júlio Torres:** Validation, Formal analysis, Investigation. **Ana Teresa Caldeira:** Validation, Formal analysis, Investigation, Resources. **Inês Silva:** Validation, Formal analysis, Investigation. **Ricardo Assunção:** Conceptualization, Methodology, Data curation, Writing – review & editing, Funding acquisition. **Paula Alvito:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Manuela Ferreira:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We deeply thank Henrique Veiga-Fernandes for kindly providing the space and equipment in his laboratory to perform the experiments. We thank Marc Veldhoen for scientific discussions and for providing *Eimeria vermiformis*. We thank Professor Tor Lea and Marthe De Boevre for helpful scientific discussions. We also thank the Vivarium and Flow Cytometry platforms at the Champalimaud Centre for the Unknown. We thank Fundação para a Ciência tecnologia (FCT), Portugal. PBA and MF were supported by Fundação para a Ciência tecnologia (FCT), Portugal (PTDC/MED-TOX/28762/2017 and IF/00425/2015, respectively). This work was funded by FCT/MCTES through national funds EarlyMyco (PTDC/MED-TOX/28762/2017), by *investigator* FCT Exploratory (IF/00425/2015/CP1324/CT0001). Thanks are also due to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113596.

References

Akbari, P., Braber, S., Varasteh, S., Alizadeh, A., Garssen, J., Fink-Gremmels, J., 2017. The intestinal barrier as an emerging target in the toxicological assessment of mycotoxins. Arch. Toxicol. 91 (3), 1007–1029. https://doi.org/10.1007/s00204-016-1794-8.

Al-Jaal, B.A., Jaganjac, M., Barcaru, A., Horvatovich, P., Latiff, A., 2019. Aflatoxin, fumonisin, ochratoxin, zearalenone and deoxynivalenol biomarkers in human biological fluids: a systematic literature review, 2001–2018. Food Chem. Toxicol. 129 (January), 211–228. https://doi.org/10.1016/j.fct.2019.04.047.

Allameh, A., Saxena, M., Raj, H.G., 1989. Interaction of aflatoxin B1 metabolites with cellular macromolecules in neonatal rats receiving carcinogen through mother's milk. Carcinogenesis 10 (11), 2131–2134. https://doi.org/10.1093/CARCIN/10.11.2131.

Almeida, R.M.A., Corrêa, B., Xavier, J.G., Mallozzi, M.A.B., Gambale, W., Paula, C.R., 1996. Acute effect of aflatoxin B1 on different inbred mouse strains II. Mycopathologia 133 (1), 23–29. https://doi.org/10.1007/BF00437095.

Alshannaq, A., Yu, J.H., 2017. Occurrence, toxicity, and analysis of major mycotoxins in food. Int. J. Environ. Res. Publ. Health 14 (6). https://doi.org/10.3390/ijerph14060632.

Alvito, P., Pereira-Da-silva, L., 2022. Mycotoxin exposure during the first 1000 Days of life and its impact on children's health: a clinical overview. Toxins 14 (3). https://doi.org/10.3390/TOXINS14030189.

Andrews-Trevino, J.Y., Webb, P., Shively, G., Rogers, B., Baral, K., Davis, D., Paudel, K., Pokharel, A., Shrestha, R., Wang, J.S., Xue, K.S., Ghosh, S., 2020. Dietary determinants of aflatoxin B1-lysine adduct in pregnant women consuming a rice-dominated diet in Nepal. Eur. J. Clin. Nutr. 74 (5), 732–740. https://doi.org/10.1038/s41430-019-0554-2.

Arora, R.G., Frölén, H., Nilsson, A., 1981. Interference of mycotoxins with prenatal development of the mouse. I. Influence of aflatoxin B1, ochratoxin A and zearalenone. Acta Vet. Scand. 22 (3–4), 524–534. https://doi.org/10.1186/BF03548677.

Assunção, R., Alvito, P., Kleiveland, C.R., Lea, T.E., 2016. Characterization of in vitro effects of patulin on intestinal epithelial and immune cells. Toxicol. Lett. 250–251, 47–56. https://doi.org/10.1016/j.toxlet.2016.04.007.

Assunção, R., Martins, C., Vasco, E., Jager, A., Oliveira, C., Cunha, S.C., Fernandes, J.O., Nunes, B., Loureiro, S., Alvito, P., 2018. Portuguese children dietary exposure to multiple mycotoxins – an overview of risk assessment under MYCOMIX project. Food Chem. Toxicol. 118, 399–408. https://doi.org/10.1016/J.FCT.2018.05.040.

Assunção, R., Pinhão, M., Loureiro, S., Alvito, P., Silva, M.J., 2019. A multi-endpoint approach to the combined toxic effects of patulin and ochratoxin a in human intestinal cells. Toxicol. Lett. 313, 120–129. https://doi.org/10.1016/J.TOXLET.2019.06.002.

Augusto, C., Oliveira, F. De, Leandra, N., 2021. Effects of prenatal exposure to aflatoxin B1 : a review. Molecules 1–11.

Awuor, A.O., Yard, E., Daniel, J.H., Martin, C., Bii, C., Romoser, A., Oyugi, E., Elmore, S., Amwayi, S., Vulule, J., Zitomer, N.C., Rybak, M.E., Phillips, T.D., Montgomery, J.M., Lewis, L.S., 2017. Evaluation of the efficacy, acceptability and palatability of calcium montmorillonite clay used to reduce aflatoxin B1 dietary exposure in a crossover study in Kenya. Food Addit. Contam. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment 34 (1), 93–102. https://doi.org/10.1080/19440049.2016.1224933.

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Rogers, H.S., Kieszak, S., Njapau, H., Schleicher, R., McCoy, L.F., Misore, A., DeCock, K., Rubin, C., Slutsker, L., Nyamongo, J., Njuguna, C., Muchiri, E., Njau, J., Maingi, S., Njoroge, J., Mutiso, J., et al., 2005. Case–control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ. Health Perspect. 113 (12), 1779. https://doi.org/10.1289/EHP.8384.

Battilani, P., Toscano, P., Van Der Fels-Klerx, H.J., Moretti, A., Camardo Leggieri, M., Brera, C., Rortais, A., Goumperis, T., Robinson, T., 2016. Aflatoxin B1 contamination in maize in Europe increases due to climate change. Sci. Rep. 6 (1), 1–7. https://doi.org/10.1038/srep24328, 2016 6:1.

Benson, A., Pifer, R., Behrendt, C.L., Hooper, L.V., Yarovinsky, F., 2009. Gut commensal bacteria direct a protective immune response against toxoplasma gondii. Cell Host Microbe 6 (2), 187–196. https://doi.org/10.1016/j.chom.2009.06.005.

Boevre, M. De, Graniczkowska, K., Saeger, S. De, 2015. Metabolism of modified mycotoxins studied through in vitro and in vivo models: an overview. Toxicol. Lett. 233 (1), 24–28. https://doi.org/10.1016/j.toxlet.2014.12.011.

Boismenu, R., Chen, Y., Havran, W.L., 1999. The role of intraepithelial γδ T cells: a gut-feelling. Microb. Infect. 1 (3), 235–240. https://doi.org/10.1016/S1286-4579(99)80039-2.

Bondy, G.S., Pestka, J.J., 2000. Immunomodulation by fungal toxins. J. Toxicol. Environ. Health B Crit. Rev. 3 (2), 109–143. https://doi.org/10.1080/109374000281113.

Bryden, W.L., 2007. Mycotoxins in the food chain: human health implications. Asia Pac. J. Clin. Nutr. 16 (Suppl. 1), 95–101.

Buszewska-Forajta, M., 2020. Mycotoxins, invisible danger of feedstuff with toxic effect on animals. Toxicon 182 (January), 34–53. https://doi.org/10.1016/j.toxicon.2020.04.101.

Caldeira, A.T., Schiavon, N., Mauran, G., Salvador, C., Rosado, T., Mirão, J., Candeias, A., 2021. On the Biodiversity and Biodeteriogenic Activity of Microbial Communities Present in the Hypogenic Environment of the Escoural Cave, Alentejo, Portugal. https://doi.org/10.3390/coatings11020209.

Caloni, F., Stammati, A., Friggè, G., De Angelis, I., 2006. Aflatoxin M1 absorption and cytotoxicity on human intestinal in vitro model. Toxicon 47 (4), 409–415. https://doi.org/10.1016/J.TOXICON.2005.12.003.

Camelo, A., Barlow, J.L., Drynan, L.F., Neill, D.R., Ballantyne, S.J., Wong, S.H., Pannell, R., Gao, W., Wrigley, K., Sprenkle, J., McKenzie, A.N.J., 2012. Blocking IL-25 signalling protects against gut inflammation in a type-2 model of colitis by suppressing nuocyte and NKT derived IL-13. J. Gastroenterol. 47 (11), 1198–1211. https://doi.org/10.1007/s00535-012-0591-2.

Chawanthayatham, S., Thiantanawat, A., Egner, P.A., Groopman, J.D., Wogan, G.N., Croy, R.G., Essigmann, J.M., 2015. Prenatal exposure of mice to the human liver carcinogen aflatoxin B1 reveals a critical window of susceptibility to genetic change. Int. J. Cancer 136 (6), 1254–1262. https://doi.org/10.1002/ijc.29102.

Chen, X., Naehrer, K., Applegate, T.J., 2016. Interactive effects of dietary protein concentration and aflatoxin B1 on performance, nutrient digestibility, and gut health

in broiler chicks. Poultry Sci. 95 (6), 1312–1325. https://doi.org/10.3382/ps/pew022.
Chen, T., Tan, T., Zhu, W., Gong, L., Yan, Y., Li, Q., Xiao, D., Li, Y., Yang, X., Hao, L., Wang, H., Yang, N., Wei, S., 2022. Exposure assessment of urinary deoxynivalenol in pregnant women in Wuhan, China. Food Chem. Toxicol. 167, 113289 https://doi.org/10.1016/J.FCT.2022.113289.
Coles, M., Veiga-Fernandes, H., 2013. Insight into lymphoid tissue morphogenesis. Immunol. Lett. 156 (1–2), 46–53. https://doi.org/10.1016/J.IMLET.2013.08.001.
Cusumano, V., Costa, G.B., Seminara, S., 1990. Effect of aflatoxins on rat peritoneal macrophages. Appl. Environ. Microbiol. 56 (11), 3482–3484. https://doi.org/10.1128/aem.56.11.3482-3484.1990.
De Agüero, M.G., Ganal-Vonarburg, S.C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H., Steinert, A., Heikenwalder, M., Hapfelmeier, S., Sauer, U., McCoy, K.D., Macpherson, A.J., 2016. The maternal microbiota drives early postnatal innate immune development. Science 351 (6279), 1296–1302. https://doi.org/10.1126/science.aad2571.
Deng, J., Zhao, L., Zhang, N.Y., Karrow, N.A., Krumm, C.S., Qi, D.S., Sun, L.H., 2018. Aflatoxin B1 metabolism: regulation by phase I and II metabolizing enzymes and chemoprotective agents. Mutat. Res. Rev. Mutat. Res. 778, 79–89. https://doi.org/10.1016/J.MRREV.2018.10.002.
Devkota, S., Wang, Y., Musch, M.W., Leone, V., Fehlner-Peach, H., Nadimpalli, A., Antonopoulos, D.A., Jabri, B., Chang, E.B., 2012. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/− mice. Nature 487 (7405), 104–108. https://doi.org/10.1038/nature11225.
Dietert, R.R., Bloom, S.E., Qureshi, M.A., Nanna, U.C., 1983. Hematological toxicology following embryonic exposure to aflatoxin-B1. In: Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology *and Medicine (New York, N.Y.)*, vol. 173, pp. 481–485. https://doi.org/10.3181/00379727-173-41674, 4.
Dietert, R.R., Qureshi, M.A., Nanna, U.C., Bloom, S.E., 1985. Embryonic exposure to aflatoxin-B1: mutagenicity and influence on development and immunity. Environ. Mutagen. 7 (5), 715–725. https://doi.org/10.1002/em.2860070510.
2002, U Doi, A., Patterson, P., Applied, E.G.-T., 2002. Variability in aflatoxin B1–macromolecular binding and relationship to biotransformation enzyme expression in human prenatal and adult liver. Elsevier. https://www.sciencedirect.com/science/article/pii/S0041008X02993993?casa_token=20ilIUlO6nUAAAAA:F5U7A00nVvNcpF8SpMc3FrHdpej0tLts-ST2MQTuq6lwHBAUhHGjAMnLjAgLwgnrByS4mbEa.
Duchenne-Moutien, R.A., Neetoo, H., 2021. Climate change and emerging food safety issues: a review. J. Food Protect. 84 (11), 1884–1897. https://doi.org/10.4315/JFP-21-141.
El-Nahla, S.M., Imam, H.M., Moussa, E.A., Ibrahim, A.M., Ghanam, A.R., 2013. Teratogenic effects of aflatoxin in Rabbits (Oryctolagus cuniculus). J. Veter. Anatomy. 6 (2), 67–85. https://doi.org/10.21608/JVA.2013.45024.
Elinav, E., Strowig, T., Kau, A.L., Henao-Mejia, J., Thaiss, C.A., Booth, C.J., Peaper, D.R., Bertin, J., Eisenbarth, S.C., Gordon, J.I., Flavell, R.A., 2011. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 145 (5), 745–757. https://doi.org/10.1016/J.CELL.2011.04.022.
Eskola, M., Kos, G., Elliott, C.T., Hajšlová, J., Mayar, S., Krska, R., 2020. Worldwide contamination of food-crops with mycotoxins: validity of the widely cited 'FAO estimate' of 25. Crit. Rev. Food Sci. Nutr. 60 (16), 2773–2789. https://doi.org/10.1080/10408398.2019.1658570.
Ferreira, M., Veiga-Fernandes, H., 2014. Pre-birth world and the development of the immune system: mum's diet affects our adult health: new insight on how the diet during pregnancy permanently influences offspring health and immune fitness Prospects & Overviews M. Ferreira and H. Veiga-Ferna. Bioessays 36 (12). https://doi.org/10.1002/bies.201400115.
Ferreira, C., Barros, L., Baptista, M., Blankenhaus, B., Barros, A., Figueiredo-Campos, P., Konjar, Š., Lainé, A., Kamenjarin, N., Stojanovic, A., Cerwenka, A., Probst, H.C., Marie, J.C., Veldhoen, M., 2020. Type 1 Treg cells promote the generation of CD8+ tissue-resident memory T cells. Nat. Immunol. 21 (7), 766–776. https://doi.org/10.1038/s41590-020-0674-9, 2020 21:7.
Fetaih, H.A., Dessouki, A.A., Hassanin, A.A.I., Tahan, A.S., 2014. Toxopathological and cytogenetic effects of aflatoxin B1 (AFB1) on pregnant rats. Pathol. Res. Pract. 210 (12), 1079–1089. https://doi.org/10.1016/j.prp.2014.06.001.
Figueiredo-Campos, P., Ferreira, C., Blankenhaus, B., Veldhoen, M., 2018. Eimeria vermiformis infection model of murine small intestine. Bio-Protocol 8 (24). https://doi.org/10.21769/BIOPROTOC.3122.
Filazi, A., Sireli, U.T., 2013. Occurrence of aflatoxins in food. Aflatoxins - Recent Adv. Future Prospects. https://doi.org/10.5772/51031.
Frank, D.N., Amand, St, A, L., Feldman, R.A., Boedeker, E.C., Harpaz, N., Pace, N.R., 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. U.S.A. 104 (34), 13780 https://doi.org/10.1073/PNAS.0706625104.
Gao, Y., Li, S., Wang, J., Luo, C., Zhao, S., Zheng, N., 2017. Modulation of intestinal epithelial permeability in differentiated caco-2 cells exposed to aflatoxin M1 and ochratoxin A individually or collectively, 2018 Toxins 10 (1), 13. https://doi.org/10.3390/TOXINS10010013. *Page 13*, 10.
Geirnaert, A., Calatayud, M., Grootaert, C., Laukens, D., Devriese, S., Smagghe, G., De Vos, M., Boon, N., Van De Wiele, T., 2017. Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. Sci. Rep. 7 (1), 1–14. https://doi.org/10.1038/s41598-017-11734-8.
Ghosh, R.C., Chauhan, H.V.S., Jha, G.J., 1991. Suppression of cell-mediated immunity by purified aflatoxin B1 in broiler chicks. Vet. Immunol. Immunopathol. 28 (2), 165–172. https://doi.org/10.1016/0165-2427(91)90138-3.
Githang'a, D., Wangia, R.N., Mureithi, M.W., Wandiga, S.O., Mutegi, C., Ogutu, B., Agweyu, A., Wang, J.S., Anzala, O., 2019. The effects of aflatoxin exposure on Hepatitis B-vaccine induced immunity in Kenyan children. Curr. Probl. Pediatr. Adolesc. Health Care 49 (5), 117–130. https://doi.org/10.1016/j.cppeds.2019.04.005.
Godfrey, K.M., Gluckman, P.D., Hanson, M.A., 2010. Developmental origins of metabolic disease: life course and intergenerational perspectives. Trends Endocrinol. Metabol. 21 (4), 199–205. https://doi.org/10.1016/J.TEM.2009.12.008.
Gong, Y.Y., Watson, S., Routledge, M.N., 2016. Aflatoxin exposure and associated human health effects, a review of epidemiological studies. Food Safety 4 (1), 14–27. https://doi.org/10.14252/foodsafetyfscj.2015026.
Goverse, G., Labao-Almeida, C., Ferreira, M., Molenaar, R., Wahlen, S., Konijn, T., Koning, J., Veiga-Fernandes, H., Mebius, R.E., 2016. Vitamin A controls the presence of RORγ^{+} innate lymphoid cells and lymphoid tissue in the small intestine. J. Immunol. 196 (12), 5148. LP – 5155.
Groopman, J.D., Egner, P.A., Schulze, K.J., Wu, L.S.F., Merrill, R., Mehra, S., Shamim, A. A., Ali, H., Shaikh, S., Gernand, A., Khatry, S.K., LeClerq, S.C., West, K.P., Christian, P., 2014. Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B1-lysine albumin biomarkers. Food Chem. Toxicol. 74, 184–189. https://doi.org/10.1016/J.FCT.2014.09.016.
Grosu, I.A., Pistol, G.C., Taranu, I., Marin, D.E., 2019. The impact of dietary grape seed meal on healthy and aflatoxin B1 afflicted microbiota of pigs after weaning. Toxins 1–16. https://doi.org/10.3390/toxins11010025.
Guerre, P., 2020. Mycotoxin and gut microbiota interactions. Toxins 12 (12), 1–33. https://doi.org/10.3390/toxins12120769.
Harvey, R.B., Huff, W.E., Kubena, L.F., Corrier, D.E., Phillips, T.D., 1988. Progression of aflatoxicosis in growing barrows. Am. J. Vet. Res. 49 (4), 482–487. https://europepmc.org/article/med/3377306.
He, Y., Fang, J., Peng, X., Cui, H., Zuo, Z., Deng, J., Chen, Z., Geng, Y., Lai, W., Shu, G., Tang, L., 2014a. Effects of sodium selenite on aflatoxin B1-induced decrease of ileal IgA+ cell numbers and immunoglobulin contents in broilers. Biol. Trace Elem. Res. 160 (1), 49–55. https://doi.org/10.1007/S12011-014-0035-3/TABLES/2.
He, Y., Fang, J., Peng, X., Cui, H., Zuo, Z., Deng, J., Chen, Z., Lai, W., Shu, G., Tang, L., 2014b. Effects of sodium selenite on aflatoxin B1-induced decrease of ileac T cell and the mRNA contents of IL-2, IL-6, and TNF-α in broilers. Biol. Trace Elem. Res. 159 (1–3), 167–173. https://doi.org/10.1007/S12011-014-9999-2/TABLES/4.
Hernández, A.J.R., E, 1996. In situ absorption of aflatoxins in rat small intestine. Mycopathologia 27–30.
Hong, Y.H., Lillehoj, H.S., Lillehoj, E.P., Lee, S.H., 2006. Changes in immune-related gene expression and intestinal lymphocyte subpopulations following Eimeria maxima infection of chickens. Vet. Immunol. Immunopathol. 114 (3), 259–272. https://doi.org/10.1016/j.vetimm.2006.08.006.
Hoytema van Konijnenburg, D.P., Reis, B.S., Pedicord, V.A., Farache, J., Victora, G.D., Mucida, D., 2017. Intestinal epithelial and intraepithelial T cell crosstalk mediates a dynamic response to infection. Cell 171 (4), 783–794. https://doi.org/10.1016/J.CELL.2017.08.046 e13.
Hsieh, C., Macatonia, S.E., Tripp, C.S., Wolf, S.F., Garra, A.O., Murphy, K.M., 1993. Development of TH1 CD4 ' T cells through IL-1 2 produced by listeria-lnduced macrophages. Science 12.
IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012. Chemical agents and related occupations. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 100 (Pt F), 9–562.
Ishikawa, A.T., Weese, J.S., Bracarense, A.P.F.R.L., Alfieri, A.A., Oliveira, G.G., Kawamura, O., Hirooka, E.Y., Veterinary, P., Londrina, U. E. De, Box, P.O., 2017. Single aflatoxin B 1 exposure induces changes in gut microbiota community in C57Bl/6 mice. World Mycotoxin J. 10 (3), 249–254. https://doi.org/10.3920/WMJ2017.2190.
Ivanovics, B., Gazsi, G., Reining, M., Berta, I., Poliska, S., Toth, M., Domokos, A., Nagy, B., Staszny, A., Cserhati, M., Csosz, E., Bacsi, A., Csenki-Bakos, Z., Acs, A., Urbanyi, B., Czimmerer, Z., 2021. Embryonic exposure to low concentrations of aflatoxin B1 triggers global transcriptomic changes, defective yolk lipid mobilization, abnormal gastrointestinal tract development and inflammation in zebrafish. J. Hazard Mater. 416 (January), 125788 https://doi.org/10.1016/j.jhazmat.2021.125788.
Jafari, T., Fallah, A.A., Kheiri, S., Fadaei, A., 2017. Food Additives & Contaminants : Part B Aflatoxin M 1 in human breast milk in Shahrekord , Iran and association with dietary factors. Food Addit. Contam. B. https://doi.org/10.1080/19393210.2017.1282545, 0(0.
Jallow, A., Xie, H., Tang, X., Qi, Z., Li, P., 2021. Worldwide aflatoxin contamination of agricultural products and foods: from occurrence to control. Compr. Rev. Food Sci. Food Saf. 20 (3), 2332–2381. https://doi.org/10.1111/1541-4337.12734.
Jha, A., Krithika, R., Manjeet, D., Verma, R.J., 2013. Protective effect of black tea infusion on aflatoxin-induced hepatotoxicity in mice. J. Clin. Exper.Hepatol. 3 (1), 29. https://doi.org/10.1016/J.JCEH.2012.12.003.
Jiang, M., Peng, X., Fang, J., Cui, H., Yu, Z., Chen, Z., 2015. Effects of aflatoxin B1 on t-cell subsets and mRNA expression of cytokines in the intestine of broilers. Int. J. Mol. Sci. 16 (4), 6945–6959. https://doi.org/10.3390/ijms16046945.
Joens, L.A., Pier, A.C., Cutlip, R.C., 1981. Effects of aflatoxin consumption on the clinical course of swine dysentery. Am. J. Vet. Res. 42 (7), 1170–1172.
Jones, L.A., Roberts, F., Nickdel, M.B., Brombacher, F., McKenzie, A.N.J., Henriquez, F. L., Alexander, J., Roberts, C.W., 2010. IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with Toxoplasma gondii. Eur. J. Immunol. 40 (2), 426–436. https://doi.org/10.1002/eji.200939705.
Kachapulula, P.W., Akello, J., Bandyopadhyay, R., Cotty, P.J., 2017. Aflatoxin contamination of groundnut and maize in Zambia: observed and potential

concentrations. J. Appl. Microbiol. 122 (6), 1471–1482. https://doi.org/10.1111/jam.13448.
Kamika, I., Takoy, L.L., 2011. Natural occurrence of aflatoxin B1 in peanut collected from kinshasa, democratic republic of Congo. Food Control 22 (11), 1760–1764. https://doi.org/10.1016/J.FOODCONT.2011.04.010.
Karlsson, O., Kim, R., Hasman, A., Subramanian, S.V., 2022. Age distribution of all-cause mortality among children younger than 5 Years in low- and middle-income countries. JAMA Netw. Open 5 (5), e2212692. https://doi.org/10.1001/JAMANETWORKOPEN.2022.12692.
Kim, S., Kim, H., Yim, Y.S., Ha, S., Atarashi, K., Tan, T.G., Longman, R.S., Honda, K., Littman, D.R., Choi, G.B., Huh, J.R., 2017. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. Nature 549 (7673), 528–532. https://doi.org/10.1038/NATURE23910.
Kim, E., Paik, D., Ramirez, R.N., Biggs, D.G., Park, Y., Kwon, H.K., Choi, G.B., Huh, J.R., 2022. Maternal gut bacteria drive intestinal inflammation in offspring with neurodevelopmental disorders by altering the chromatin landscape of CD4+ T cells. Immunity 55 (1), 145–158. https://doi.org/10.1016/J.IMMUNI.2021.11.005 e7.
Lacroix, D., Sonnier, M., Moncion, A., Cheron, G., Cresteil, T., 1997. Expression of CYP3A in the human liver - evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. Eur. J. Biochem. 247 (2), 625–634. https://doi.org/10.1111/J.1432-1033.1997.00625.X.
Lamplugh, S., Hendrickse, R., Apeagyei, F., Mwanmut, D., 1988. Aflatoxins in breast milk, neonatal cord blood, and serum ofpregnant women. Br. Med. J. 296, 968.
Lauer, J.M., Jeffrey, K., Agaba, E., Duggan, C.P., Ausman, L.M., Kathy, S., Nshakira, N., Ghosh, S., 2019. Maternal aflatoxin exposure during pregnancy and adverse birth outcomes in Uganda. Matern. Child Nutr. 1–7. https://doi.org/10.1111/mcn.12701. March 2018.
Liew, W.P.P., Mohd-Redzwan, S., 2018. Mycotoxin: its impact on gut health and microbiota. Front. Cell. Infect. Microbiol. 8 (FEB), 60. https://doi.org/10.3389/FCIMB.2018.00060/BIBTEX.
Lu, C., Yan, Y., Jian, F., Ning, C., 2021. Coccidia-microbiota interactions and their effects on the host. Front. Cell. Infect. Microbiol. 11 (October), 1–12. https://doi.org/10.3389/fcimb.2021.751481.
Ma, H., Tao, W., Zhu, S., 2019. T lymphocytes in the intestinal mucosa: defense and tolerance. Cell. Mol. Immunol. 16 (3), 216–224. https://doi.org/10.1038/s41423-019-0208-2, 2019 16:3.
Magan, N., Olsen, M., 2004. Mycotoxins in food: detection and control. In: *Mycotoxins In Food: Detection And Control* (Issue March). https://doi.org/10.1533/9781855739086.
Marin, D.E., Taranu, I., Bunaciu, R.P., Pascale, F., Tudor, D.S., Avram, N., Sarca, M., Cureu, I., Criste, R.D., Suta, V., Oswald, I.P., 2002. Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin. J. Anim. Sci. 80 (5), 1250–1257. https://doi.org/10.2527/2002.8051250X.
Martins, C., Assunção, R., Cunha, S.C., Fernandes, J.O., Jager, A., Petta, T., Augusto, C., Alvito, P., 2018. Assessment of multiple mycotoxins in breakfast cereals available in the Portuguese market. Food Chem. 239, 132–140. https://doi.org/10.1016/j.foodchem.2017.06.088.
McKenzie, G.J., Bancroft, A., Grencis, R.K., McKenzie, A.N.J., 1998. A distinct role for interleukin-13 in Th2-cell-mediated immune responses. Curr. Biol. 8 (6), 339–342. https://doi.org/10.1016/S0960-9822(98)70134-4.
2006, U Meissonnier, G., Marin, D., Galtier, P., G, B.-N., 2006. Modulation of the immune response by a group of fungal food contaminant, the aflatoxins. In: Nutrition and Immunity, pp. 147–166. ref.92). https://www.cabdirect.org/cabdirect/abstract/20063204204.
Meissonnier, G.M., Pinton, P., Laffitte, J., Cossalter, A.M., Gong, Y.Y., Wild, C.P., Bertin, G., Galtier, P., Oswald, I.P., Laf, J., Cossalter, A.M., Yun, Y., Wild, C.P., Bertin, G., Galtier, P., Oswald, I.P., 2008. Immunotoxicity of aflatoxin B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. Toxicol. Appl. Pharmacol. 231 (2), 142–149. https://doi.org/10.1016/J.TAAP.2008.04.004.
Methenitou, G., Maravelias, C., Athanaselis, S., Dona, A., Koutselinis, A., 2001. Immunomodulative effects of aflatoxins and selenium on human natural killer cells. Vet. Hum. Toxicol. 43 (4), 232–234. http://europepmc.org/abstract/MED/11474740.
Mocchegiani, E., Corradi, A., Santarelli, L., Tibaldi, A., Deangelis, E., Borghetti, P., Bonomi, A., Fabris, N., Cabassi, E., 1998. Zinc, thymic endocrine activity and mitogen responsiveness (PHA) in piglets exposed to maternal aflatoxicosis B1 and G1. Vet. Immunol. Immunopathol. 62 (3), 245–260. https://doi.org/10.1016/S0165-2427(98)00073-7.
Monticelli, L.A., Osborne, L.C., Noti, M., Tran, S.V., Zaiss, D.M.W., Artis, D., 2015. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. Proc. Natl. Acad. Sci. U.S.A. 112 (34), 10762–10767. https://doi.org/10.1073/PNAS.1509070112.
Muñoz, M., Eidenschenk, C., Ota, N., Wong, K., Lohmann, U., Kühl, A.A., Wang, X., Manzanillo, P., Li, Y., Rutz, S., Zheng, Y., Diehl, L., Kayagaki, N., van Lookeren-Campagne, M., Liesenfeld, O., Heimesaat, M., Ouyang, W., 2015. Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection. Immunity 42 (2), 321–331. https://pubmed.ncbi.nlm.nih.gov/25680273/.
Neldon-Ortiz, D.L., Qureshi, M.A., 1992. Effects of AFB1 embryonic exposure on chicken mononuclear phagocytic cell functions. Dev. Comp. Immunol. 16 (2), 187–196. https://doi.org/10.1016/0145-305X(92)90018-8.
Ngo, P.A., Neurath, M.F., López-Posadas, R., 2022. Impact of epithelial cell shedding on intestinal homeostasis. Int. J. Mol. Sci. 23 (8) https://doi.org/10.3390/IJMS23084160.
Nishida, A., Inoue, R., Inatomi, O., Bamba, S., Naito, Y., Andoh, A., 2018. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin. J. Gastroenterol. 11 (1) https://doi.org/10.1007/S12328-017-0813-5.
Okamura, H., Tsutsui, H., Komatsu, T., Yutsudo, M., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., Akita, K., Namba, M., Tanabe, F., Konishi, K., Fukuda, S., Kurimoto, M., 1995. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 378 (6552), 88–91. https://doi.org/10.1038/378088A0.
Omata, Y., Frech, M., Primbs, T., Lucas, S., Andreev, D., Scholtysek, C., Sarter, K., Kindermann, M., Yeremenko, N., Baeten, D.L., Andreas, N., Kamradt, T., Bozec, A., Ramming, A., Krönke, G., Wirtz, S., Schett, G., Zaiss, M.M., 2018. Group 2 innate lymphoid cells attenuate inflammatory arthritis and protect from bone destruction in mice. Cell Rep. 24 (1), 169–180. https://doi.org/10.1016/j.celrep.2018.06.005.
O'Connor, W., Kamanaka, M., Booth, C.J., Town, T., Nakae, S., Iwakura, Y., Kolls, J.K., Flavell, R.A., 2009. A protective function for interleukin 17A in T cell-mediated intestinal inflammation. Nat. Immunol. 10 (6), 603–609. https://doi.org/10.1038/NI.1736.
Panangala, V.S., Giambrone, J.J., Diener, U.L., Davis, N.D., Hoerr, F.J., Mitra, A., Schultz, R.D., Wilt, G.R., 1986. Effects of aflatoxin on the growth performance and immune responses of weanling swine. Am. J. Vet. Res. 47 (9), 2062–2067. https://europepmc.org/article/med/3767113.
Panda, S.K., Colonna, M., 2019. Innate lymphoid cells in mucosal immunity. Front. Immunol. 10 (MAY), 1–13. https://doi.org/10.3389/fimmu.2019.00861.
Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.-H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., Dong, C., 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol. 6 (11), 1133–1141. https://doi.org/10.1038/ni1261.
Park, S.H., Kim, D., Kim, J., Moon, Y., 2015. Effects of mycotoxins on mucosal microbial infection and related pathogenesis. Toxins 7 (11), 4484–4502. https://doi.org/10.3390/toxins7114484.
Partanen, H.A., El-Nezami, H.S., Leppänen, J.M., Myllynen, P.K., Woodhouse, H.J., Vähäkangas, K.H., 2009. Aflatoxin B1 transfer and metabolism in human placenta. Toxicol. Sci. 113 (1), 216–225. https://doi.org/10.1093/toxsci/kfp257.
Payros, D., Alassane-Kpembi, I., Pierron, A., Loiseau, N., Pinton, P., Oswald, I.P., 2016. Toxicology of deoxynivalenol and its acetylated and modified forms. Arch. Toxicol. 90 (12), 2931–2957. https://doi.org/10.1007/s00204-016-1826-4.
Pickard, J.M., Maurice, C.F., Kinnebrew, M.A., Abt, M.C., Schenten, D., Golovkina, T.V., Bogatyrev, S.R., Ismagilov, R.F., Pamer, E.G., Turnbaugh, P.J., Chervonsky, A.V., 2014. Rapid fucosylation of intestinal epithelium sustains host–commensal symbiosis in sickness. Nature 514 (7524), 638–641. https://doi.org/10.1038/nature13823.
Potchinsky, M.B., Bloom, S.E., 1993. Selective aflatoxin B1-induced sister chromatid exchanges and cytotoxicity in differentiating B and T lymphocytes in vivo. Environ. Mol. Mutagen. 21 (1), 87–94. https://doi.org/10.1002/em.2850210112.
Qian, G., Tang, L., Guo, X., Wang, F., Massey, M.E., Su, J., Guo, T.L., Williams, J.H., Phillips, T.D., Wang, J.S., 2014. Aflatoxin B1 modulates the expression of phenotypic markers and cytokines by splenic lymphocytes of male F344 rats. J. Appl. Toxicol. : JAT (J. Appl. Toxicol.) 34 (3), 241. https://doi.org/10.1002/JAT.2866.
Qiu, Z., Sheridan, B.S., 2018. Isolating lymphocytes from the mouse small intestinal immune system. JoVE 132, e57281. https://doi.org/10.3791/57281.
Qureshi, M.A., Brake, J., Hamilton, P.B., Hagler, W.M., Nesheim, S., 1998. Dietary exposure of broiler breeders to aflatoxin results in immune dysfunction in progeny chicks. Poultry Sci. 77 (6), 812–819. https://doi.org/10.1093/ps/77.6.812.
Raiola, A., Tenore, G.C., Manyes, L., Meca, G., Ritieni, A., 2015. Risk analysis of main mycotoxins occurring in food for children: an overview. Food Chem. Toxicol. 84, 169–180. https://doi.org/10.1016/J.FCT.2015.08.023.
Raisuddin, Singh, K.P., Zaidi, S.I.A., Saxena, A.K., Ray, P.K., 1990. Effects of aflatoxin on lymphoid cells of weanling rat. J. Appl. Toxicol. 10 (4), 245–250. https://doi.org/10.1002/jat.2550100404.
Reddy, R.V., Taylor, M.J., Sharma, R.P., 1987. Studies of immune function of CD-1 mice exposed to aflatoxin B1. Toxicology 43 (2), 123–132. https://doi.org/10.1016/0300-483X(87)90002-3.
Roberts, S.J., Smith, A.L., West, A.B., Wen, L., Findly, R.C., Owen, M.J., Hayday, A.C., 1996. T-cell alpha beta + and gamma delta + deficient mice display abnormal but distinct phenotypes toward a natural, widespread infection of the intestinal epithelium. Proc. Natl. Acad. Sci. USA 93 (21), 11774–11779. https://doi.org/10.1073/pnas.93.21.11774.
Rushing, B.R., Selim, M.I., 2019. Aflatoxin B1: a review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food Chem. Toxicol. 124 (November 2018), 81–100. https://doi.org/10.1016/j.fct.2018.11.047.
Scott, T.R., Rowland, S.M., Rodgers, R.S., Bodine, A.B., 1991. Genetic selection for aflatoxin B1 resistance influences chicken T-cell and thymocyte proliferation. Dev. Comp. Immunol. 15 (4), 383–391. https://doi.org/10.1016/0145-305X(91)90030-3.
Shields, J.M., Olson, B.H., 2003. Cyclospora cayetanensis: a review of an emerging parasitic coccidian. Int. J. Parasitol. 33 (4), 371–391. https://doi.org/10.1016/S0020-7519(02)00268-0.
Shuaib, F.M.B., Jolly, P.E., Ehiri, J.E., Yatich, N., Jiang, Y., Funkhouser, E., Person, S.D., Wilson, C., Ellis, W.O., Wang, J.S., Williams, J.H., 2010. Association between birth outcomes and aflatoxin B1 biomarker blood levels in pregnant women in Kumasi, Ghana. Trop. Med. Int. Health 15 (2), 160–167. https://doi.org/10.1111/j.1365-3156.2009.02435.x.
Silvotti, L., Petterino, C., Bonomi, A., Cabassi, E., 1997. Immunotoxicological Effects on Piglets of Feeding Sows Diets Containing Aflatoxins. Veterinary Record.
Singh, R.P., 2019. Glycan utilisation system in Bacteroides and Bifidobacteria and their roles in gut stability and health. Appl. Microbiol. Biotechnol. 103 (18), 7287–7315. https://doi.org/10.1007/S00253-019-10012-Z.

Stange, J., Hepworth, M.R., Rausch, S., Zajic, L., Kühl, A.A., Uyttenhove, C., Renauld, J.-C., Hartmann, S., Lucius, R., 2012. IL-22 mediates host defense against an intestinal intracellular parasite in the absence of IFN-γ at the cost of Th17-driven immunopathology. J. Immunol. 188 (5), 2410–2418. https://doi.org/10.4049/jimmunol.1102062.

Stras, S.F., Werner, L., Toothaker, J.M., Rechavi, E., Shouval, D.S., Correspondence, L.K., 2019. Maturation of the human intestinal immune system occurs early in fetal development. Dev. Cell 51, 357–373. https://doi.org/10.1016/j.devcel.2019.09.008 e5.

Tesfamariam, K., Argaw, A., Hanley-cook, G.T., Gebreyesus, S.H., Kolsteren, P., Belachew, T., Velde, M. Van De, Saeger, S. De, Boevre, M. De, Lachat, C., 2022. Multiple mycotoxin exposure during pregnancy and risks of adverse birth outcomes : a prospective cohort study in rural Ethiopia. Environ. Int. 160 (December 2021), 107052 https://doi.org/10.1016/j.envint.2021.107052.

Thompson, E.A., Mitchell, J.S., Beura, L.K., Torres, D.J., Mrass, P., Pierson, M.J., Cannon, J.L., Masopust, D., Fife, B.T., Vezys, V., 2019. Interstitial migration of CD8αβ T cells in the small intestine is dynamic and is dictated by environmental cues. Cell Rep. 26 (11), 2859–2867. https://doi.org/10.1016/J.CELREP.2019.02.034 e4.

Tierney, J., Gowing, H., Van Sinderen, D., Flynn, S., Stanley, L., McHardy, N., Hallahan, S., Mulcahy, G., 2004. In vitro inhibition of Eimeria tenella invasion by indigenous chicken Lactobacillus species. Vet. Parasitol. 122 (3), 171–182. https://doi.org/10.1016/j.vetpar.2004.05.001.

Torow, N., Marsland, B.J., Hornef, M.W., Gollwitzer, E.S., 2016. Neonatal mucosal immunology. Mucosal Immunol. 10 (1), 5–17. https://doi.org/10.1038/mi.2016.81, 2017 10:1.

Van De Pavert, S.A., Ferreira, M., Domingues, R.G., Ribeiro, H., Molenaar, R., Moreira-Santos, L., Almeida, F.F., Ibiza, S., Barbosa, I., Goverse, G., Labão-Almeida, C., Godinho-Silva, C., Konijn, T., Schooneman, D., Oà Toole, T., Mizee, M.R., Habani, Y., Haak, E., Santori, F.R., et al., 2014. Maternal retinoids control type 3 innate lymphoid cells and set the offspring immunity. Nature 508 (7494), 123. https://doi.org/10.1038/NATURE13158.

Venturini, M.C., Perfumo, C.J., Risso, M.A., Gomez, C.M., Piscopo, M.V., 1990. Effect of aflatoxin B 1 on resistance induced by Bordetella bronchiseptica vaccine in rabbits. Vet. Microbiol. 25.

Venturini, M.C., Quiroga, M.A., Risso, M.A., Lorenzo, C. Di, Omata, Y., Venturini, L., Godoy, H., 1996. Mycotoxin T-2 and aflatoxin B 1 as immunosuppressors in mice chronically infected with toxoplasma gondii. J. Comp. Pathol. 115, 229–237.

Wang, J., Tang, L., Glenn, T.C., Wang, J.S., 2016. Aflatoxin B1 induced compositional changes in gut microbial communities of male F344 rats. Toxicol. Sci. : Off. J. Soc. Toxicol. 150 (1), 54–63. https://doi.org/10.1093/TOXSCI/KFV259.

Wang, L., Huang, Q., Wu, J., Wu, W., Jiang, J., Yan, H., Huang, J., 2022. The metabolism and biotransformation of AFB 1 : key enzymes and pathways. Biochem. Pharmacol. 199 (March) https://doi.org/10.1016/j.bcp.2022.115005.

Wangikar, P.B., Dwivedi, P., 2005. Effects of aflatoxin B 1 on embryo fetal development in rabbits. Food Chem. Toxicol. 43, 607–615. https://doi.org/10.1016/j.fct.2005.01.004.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., Aggarwal, D., 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. 80 (5), 1106–1122. https://doi.org/10.1093/AJCN/80.5.1106.

Wogan, G.N., 1973. Aflatoxin carcinogenesis. Methods Cancer Res. 309–344.

Wu, C.Q., Gao, Y.N., Li, S.L., Huang, X., Bao, X.Y., Wang, J.Q., Zheng, N., 2019. Modulation of intestinal epithelial permeability and mucin mRNA (MUC2, MUC5AC, and MUC5B) expression and protein secretion in Caco-2/HT29-MTX co-cultures exposed to aflatoxin M1, ochratoxin A, and zearalenone individually or collectively. Toxicol. Lett. 309, 1–9. https://doi.org/10.1016/J.TOXLET.2019.03.010.

Xu, W., Domingues, R.G., Fonseca-Pereira, D., Ferreira, M., Ribeiro, H., Lopez-Lastra, S., Motomura, Y., Moreira-Santos, L., Bihl, F., Braud, V., Kee, B., Brady, H., Coles, M.C., Vosshenrich, C., Kubo, M., Di Santo, J.P., Veiga-Fernandes, H., 2015. NFIL3 Orchestrates the emergence of common helper innate lymphoid cell precursors. Cell Rep. 10 (12), 2043–2054. https://doi.org/10.1016/J.CELREP.2015.02.057/ATTACHMENT/A8C9B121-5A87-486C-89EC-D11745DC523C/MMC1.PDF.

Yang, Xiai, Liangliang Liu, J.C., A, X., 2017. Response of intestinal bacterial flora to the long-term feeding of aflatoxin B1 (AFB1) in mice. Toxins 1, 1–12. https://doi.org/10.3390/toxins9100317.

Yin, H., Jiang, M., Peng, X., Cui, H., Zhou, Y., He, M., Zuo, Z., Ouyang, P., Fan, J., Fang, J., 2016. The molecular mechanism of G2/M cell cycle arrest induced by AFB1 in the jejunum. Oncotarget 7 (24), 35592. https://doi.org/10.18632/ONCOTARGET.9594.

Yunus, A.W., Ghareeb, K., Abd-El-Fattah, A.A.M., Twaruzek, M., Böhm, J., 2011a. Gross intestinal adaptations in relation to broiler performance during chronic aflatoxin exposure. Poultry Sci. 90 (8), 1683–1689. https://doi.org/10.3382/PS.2011-01448.

Yunus, A.W., Razzazi-Fazeli, E., Bohm, J., 2011b. Aflatoxin B1 in affecting broiler's performance, immunity, and gastrointestinal tract: a review of history and contemporary issues. Toxins 3 (6), 566. https://doi.org/10.3390/TOXINS3060566.

Zhang, S., Peng, X., Fang, J., Cui, H., Zuo, Z., Chen, Z., 2014. Effects of aflatoxin B1 exposure and sodium selenite supplementation on the histology, cell proliferation, and cell cycle of jejunum in broilers. Biol. Trace Elem. Res. 160 (1), 32–40. https://doi.org/10.1007/S12011-014-0009-5.

Zhou, J., Tang, L., Wang, J.-S., 2019. Assessment of the adverse impacts of aflatoxin B(1) on gut-microbiota dependent metabolism in F344 rats. Chemosphere 217, 618–628. https://doi.org/10.1016/j.chemosphere.2018.11.044.

Zhou, J., Tang, L., Wang, J.-S., 2021. Aflatoxin B1 induces gut-inflammation-associated fecal lipidome changes in F344 rats. Toxicol. Sci. : Off. J. Soc. Toxicol. 183 (2), 363–377. https://doi.org/10.1093/toxsci/kfab096.