How maternal exposure to aflatoxin B1 (AFB1) impacts the development of progeny intestinal immune system?

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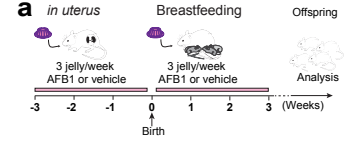
Introduction

Exposure to toxic contaminants during early-life is associated with the development of diseases. Aflatoxins can cross placental barrier and were found in breast milk leading individuals to mycotoxins exposition since early stages of life[1]. However how maternal exposure to mycotoxins influences the development and function of the offspring's immune system remains largely unexplored. Recently, we showed that in utero maternal exposure to micronutrients is critical for the development of the immune system, which sets long term immunity if the progeny[2]. Here we show that presence of aflatoxin B1 in the diet of pregnant murine females affects the development and function of the intestinal immune system.

[1] Assunção R, et al., Food Chem Toxicol, 86, 274-81.

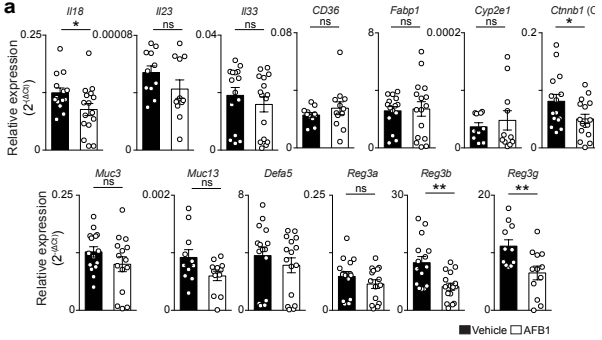
[2] van de Pavert SA, Ferreira M, et al., 2014, Nature, 508(7494), 123-127.

Experimental setting



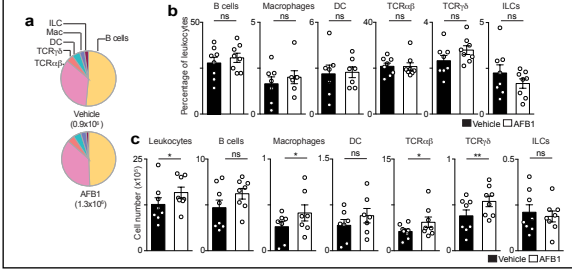
a. Schematic representation of the self-feeding schedule to administer AFB1 in jelly-pellets to females in gestation and lactation. C57Bl6/J pregnant females (E10.5) were treated with jelly pellets containing AFB1(400ug/Kg BW) or vehicle 3 times a week during gestation and breastfeeding period. Offspring litters were analysed at 4-5 week s old. Litter size and body weight of the offspring are not affected by AFB1 maternal exposure (Data not shown). The development of secondary lymphoid organs are not affected by AFB1 maternal exposure(Data not shown).

Maternal AFB1 exposure leads to downregulation of immune defense-related genes in epithelium



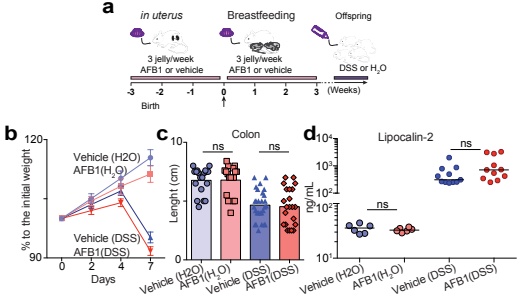
a. Relative mRNA expression of epithelial-derived genes in intestine of AFB1-exposed progeny intestine or control (vehicle) determined by quantitative PCR. Specific transcript levels were determined relative to Hprt, Gapdh mRNA levels. Mean and error bars: s.e.m. Vehicle n=11; AFB1 n=12; n represents biologically independent animals. Mann-Whitney test. Data represents 2 independent experiments. *P<0.05; **P<0.01; ns not significant.

Maternal AFB1 exposure leads to increase of intestinal innate and adaptive immune cell subsets



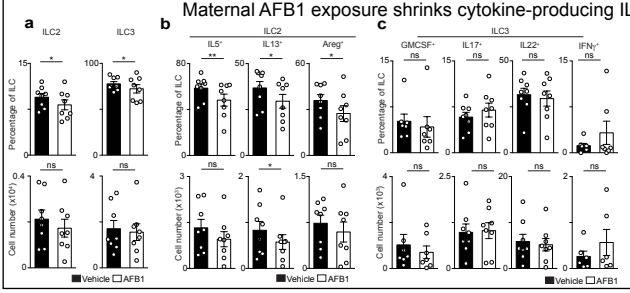
Flow cytometry analysis of major leukocytes in intestinal lamina propria. **a.** Chart graphs represent the frequency of indicated populations (Mac, macrophages; DC, dendritic cells) of all leukocytes in lamina propria. **b.** Frequency of distinct immune cell subsets of total lamina propria leukocytes. **c.** Total number of immune cells for each subset in intestinal lamina propria. Data represents 3 independent experiments. Vehicle and AFB1 n=5 to 6. n represents litters. Each litter included at least 4 animals. Data represents the summary of 2 independent experiments. Mean and error bars: s.e.m. Wilcoxon test. *P<0.05; **P<0.01; ns not significant.

AFB1 maternal-exposed progeny are not more susceptible to DSS-induced intestinal inflammation



a. Schematic representation of the self-feeding schedule to administer AFB1 in jelly-pellets to females in gestation and breastfeeding. Offspring mice received then acute treatment of DSS to induce colitis. **b.** Percentage to the initial weight upon DSS treatment. **c.** Colon length upon DSS treatment. d. Lipocalin-2 levels in serum quantified by ELISA. Vehicle and AFB1 n=6 to 12 ; n=12; represents biologically independent animals. Mann-Whitney test. Data represents 2 independent experiments. *P<0.05; **P<0.01; ns not significant.

Maternal AFB1 exposure shrinks cytokine-producing ILC2 subsets



Flow cytometry analysis of ILC subsets. **a.** Frequency and number of ILC2 and ILC3 in intestinal lamina propria. **b.** Frequency and number of cytokine-producing ILC2 subsets. **c.** Frequency and number of cytokine-producing ILC3 subsets. Data represents 3 independent experiments. Vehicle and AFB1 n=5 to 6. n represents litters. Each litter included at least 4 animals. Data represents the summary of 2 independent experiments. Mean and error bars: s.e.m. Wilcoxon test. *P<0.05; **P<0.01; ns not significant.

Acknowledgements

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