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Amoxicillin modulates the intestinal microbiota and impairs the development of oral tolerance to whey – a study in Brown Norway rats

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Background: Antibiotic use during infancy has been highlighted as a risk factor for development of allergy later in life. However, few studies have investigated the effects of antibiotics on tolerance development. In the present study we investigated how administration of the commonly used antibiotic amoxicillin (AMX) impacts on tolerance development to whey.

Method: Brown Norway (BN) rats (n=12/group) were gavaged daily with either AMX or water for 4 weeks. After one week of AMX treatment, rats were given a whey based hydrolysed infant formula (IF) *ad libitum* in drinking water for 3 weeks. Rats were subsequently post-immunised i.p. with whey once a week for 4 weeks to assess tolerance induction. Elicitation was measured by *in vivo* tests. Whey-specific IgG1 and IgE were quantified in serum, and total and specific IgA in faeces by means of ELISAs. In parallel the microbial composition of ileum, cecum and faeces was analysed by high throughput 16S rRNA gene sequencing after one week of AMX treatment. T and B cells from intestinal tissues and blood were analysed by flow cytometry.

Results: After one week of AMX treatment, corresponding to the time of tolerance induction initiation, the faecal and cecal microbiota showed significantly reduced alpha diversity and observed shifts in compositions were similar to those previously reported in atopic infants. Changes included a reduction in relative abundance of the genera *Lactobacillus and Bifidobacterium* and a general reduction in firmicutes compared to untreated controls. Conversely, the genera *Bacteroides, Escherichia* and *Klebsiella* expanded. Analysis of ileum content revealed a distinct bacterial community composition compared to cecum/faeces. While the effects of AMX generally were similar in ileum compared to cecum/faeces, some genera, including *Bifidobacterium*, was affected by AMX in the opposite direction.

The serum levels of whey-specific IgG1 and IgE after post-immunisations were higher in rats treated by AMX compared to those not treated by antibiotic. This effect persisted over time. AMX increased total faecal IgA, and increased the frequency of regulatory CD25+FoxP3+ T helper cells in lamina propria.

Conclusion: AMX impaired *de novo* tolerance induction by whey based hydrolysed IF, and resulted in higher levels of specific IgG1 and IgE after post-immunisations. AMX affected both the humoral and cellular immune system in the gut. These effects are likely due to modulation of the intestinal microbiota.