ASSESSMENT OF GASTROINTESTINAL MUCOSAL IMMUNITY IN A SPONTANEOUS MODEL OF COLITIS

INTRODUCTION

- Gut associated lymphoid tissue is part of the mucosal immune system. Abnormal immune response is one of the factors that has a major contribution in the group of diseases known as inflammatory bowel disease (IBD). A balanced interaction between pro-inflammatory and anti-inflammatory mediators like interleukin 10 (IL-10) are important to prevent disease. The relevance of this cytokine in the formation of immune responses produced in the mucosa has been proved by the spontaneous inflammation observed in the IL-10 deficient mouse. Mice knockout for IL-10 develop chronic enterocolitis by 2-3 months of age, and have been a popular model to study this group of diseases.

- Since IgA acts as a non-inflammatory immune protection factor at the intestinal mucosa surface, the objective of this study was to study the secretion pattern of sIgA during the development of spontaneous colitis in this model.

RESULTS

![Figure 1](https://example.com/figure1.png) **Figure 1**: Secretory IgA in feces, table. Feces were recovered each week from week 6 to week 18 and pooled in two groups, knockout (KO) and wild type (WT). The concentration of sIgA was measured by ELISA, after diluting feces in PBS 1:20 and then diluting 1:100.

We observe that even there is high variation in IgA secretion between weeks, it seems that secretion is increased in KO mice.

![Figure 2](https://example.com/figure2.png) **Figure 2**: Shows the evolution sIgA concentration measured with ELISA, by grouping the samples in three periods, and were represented in the graphic as means placed in the middle of this periods. First point comprises means from 6 to 10 weeks, second from 10 to 14 weeks, third from 14 to 18 weeks.

Even IgA concentration is increased in KO than WT, at 10-14 week period the slope is more pronounced.

![Figure 3](https://example.com/figure3.png) **Figure 3**: Secretory IgA concentrations in intestinal lavages in mice IL-knockout at the beginning and the end of the experiment, where each point represents an individual. The graphic shows IgA concentration of wild type and knockout mice after acclimatization and the end of this experiment. The mean concentration of WT at week 6 is 1,617±0,836, KO at week 6 is 1,617±0,836, WT at week 18 is 2,516±0,207 and KO in week 18 is 6,272±2,789. A two-way ANOVA was performed and resulting with significant differences between weeks (p=0,0016) and between animal types (p=0,0029) and also found significant the interaction between this two factors (p=0,0188).

The concentration of sIgA in KO mice is significantly different compared to WT mice, and also the deviation between samples in KO at week 18 is (6,272±2,789) greater than WT 2,516±0,207.

CONCLUSIONS

- The concentration of sIgA is increased in IL-10 knockout mice compared to those of wild type phenotype.

- Results indicate changes on IgA concentration from 12 weeks on. These results correlate well with the reported onset of the colitis in this model. However in our conditions we did not observe clinical signs.

- IgA response differed from that reported in human IBD patients since sIgA concentration on ulcerative colitis and Crohn’s disease patients tends to be reduced or equal to control healthy groups.

- Increased sIgA in feces might be a good marker for the follow up of the development of the spontaneous colitis in this mouse model.

REFERENCES


MATERIALS AND METHODS

- 10 five week old C57BL6 (WT) mice and 10 five week old B6.129P2-IL10tm1Cgn (KO) mice were obtained from Jackson Laboratories (Maine, USA) and were distributed in two groups and body weight of the animals was monitored weekly through the experiment. After acclimatization, 8 animals were anesthetized with isoﬂurane and were euthanized by cervical dislocation. Then the small intestine (SI) was recovered and divided in two halves. The distal fragment was carefully rinsed with cold sterile sodium chloride solution at 0.9% concentration, in order to remove fecal content. The SI was then cut lengthwise and was placed in plates with 2ml of phosphate buffered saline (PBS) and incubated at 37°C for 30 minutes. The wash was obtained after this and was conserved on -18°C.

- In the rest of the animals ecal samples were collected fresh weekly from first week until week 18 (a total of 12 samples per group), were diluted in PBS at 1:20 concentration and kept at -18°C until analysis as a pooled sample.

When animals were 18 week old, they were euthanized and the intestinal wash was recovered following the same procedure described before. With the first week samples, a total of 19 samples of intestinal wash were obtained.

- Fecal dilutions and intestinal washes were diluted in Assay Buffer (PBSx1) at a concentration of 1:100, previously to the analysis, and IgA levels in fecal samples and small intestine were quantified by enzyme-linked immunosorbent assay (ELISA). An ANOVA analysis was performed with the intestinal lavages using a software PRISM 7 from Graphpad software INC. Means were considered statistically different when P < 0.05.