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Unraveling the role of IL-17A in the intestinal immune response against the protozoan parasite *Giardia muris* by an RNA sequencing approach

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Introduction

The protozoan parasite *Giardia duodenalis* is a highly common intestinal pathogen with a wide vertebrate host range, including humans. *Giardia muris* is a natural parasite of rodents. *Giardia* species have a simple and direct life cycle. After infective cysts are ingested orally, excystation occurs, resulting in the release of flagellated trophozoites that attach to the mucosa of the small intestine. After some rounds of asexual reproduction, encystation takes place. The cysts are then shed with the faeces, ready to be taken up by a new host.

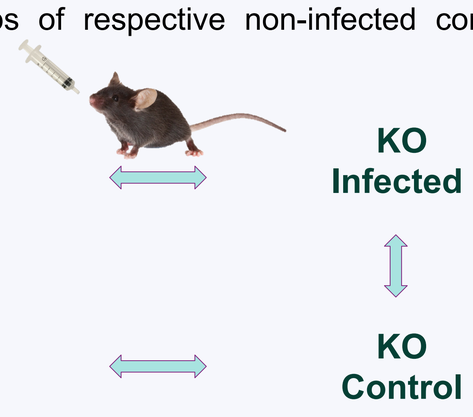
An infection with *Giardia* is in many cases acute and self-limiting. However, a significant proportion of infected hosts develop a chronic infection. In mice infected with *G. muris*, clearing of the parasite is achieved around week 3 post-infection. Transcriptional analysis of small intestinal tissue revealed strong upregulation of IL-17A after infection. Furthermore, 3 weeks post-infection, IL-17A receptor A KO mice were unable to mount a protective immune response against *G. muris.*

Aim of the study

The aim of the study was to unravel the effector mechanisms related to the protective IL-17A response against *G. muris* in mice.

Materials and Methods

C57Bl/6 WT and C57Bl/6 IL-17RA KO mice were orally infected with 10^3 *G. muris* cysts. 2 groups of respective non-infected control mice were included in the study.



3 weeks post-infection the intestinal transcriptome of the 4 groups of mice was analysed by RNA-seq. Differentially expressed genes in the 4 comparisons were identified with DESeq2 software and used as an input for Ingenuity Pathway Analysis (IPA) software in order to extract biological pathways and functions. Genes were plotted in heatmaps to visualize dominant expression patterns.

Results

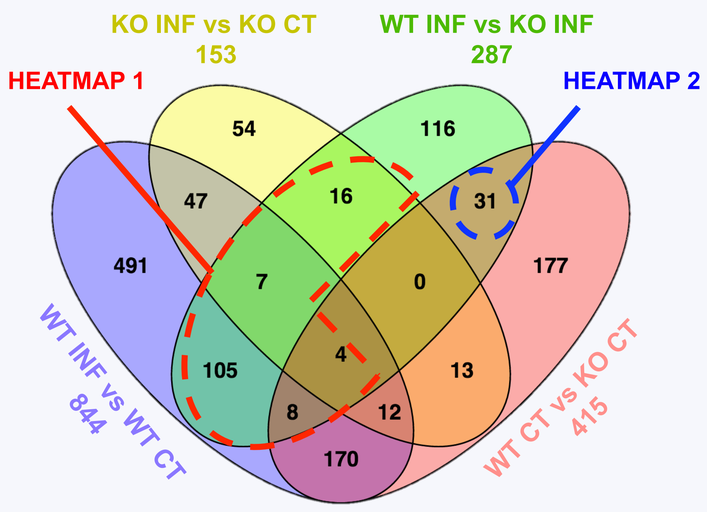


Figure 1. 844 genes were differentially expressed between WT infected and WT control mice

153 genes between KO infected and KO control mice

287 genes between WT infected and KO infected mice

415 genes between WT control and KO control mice

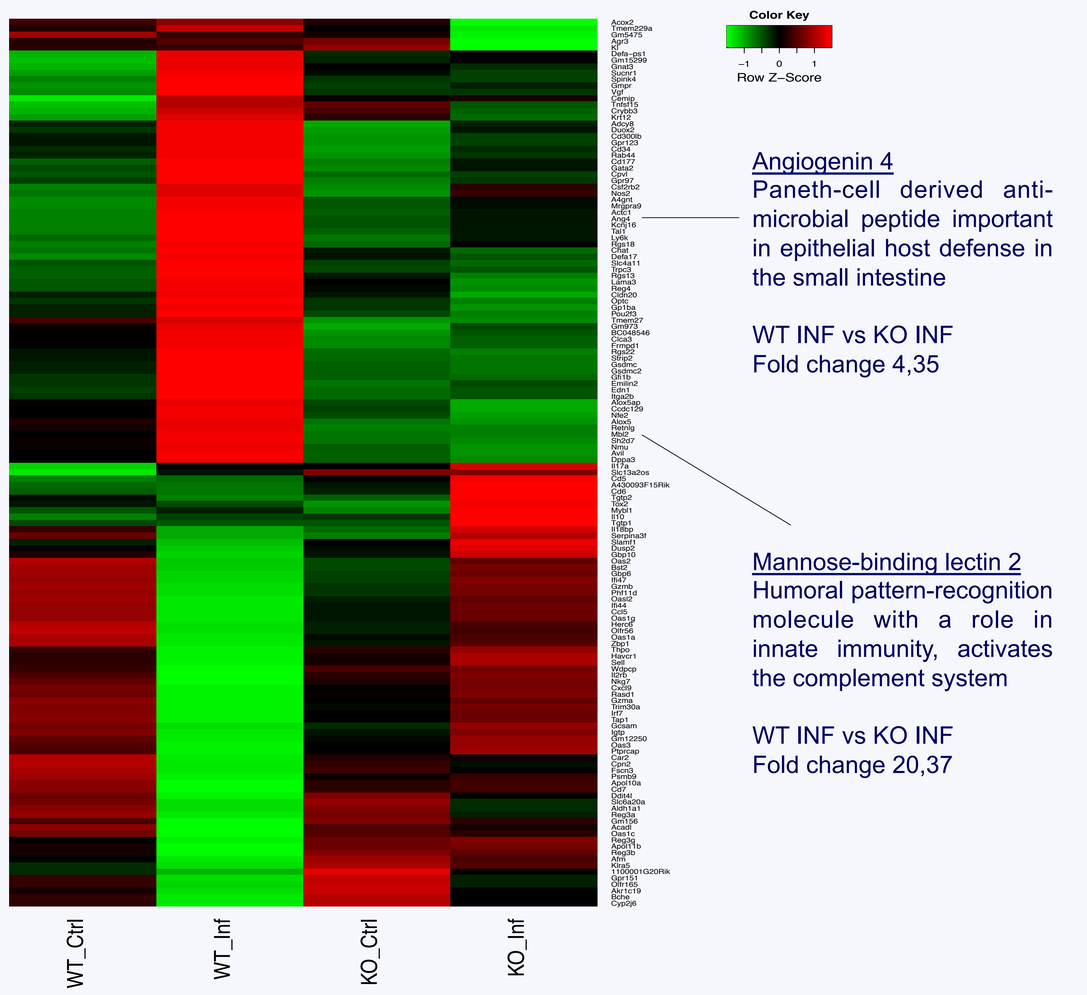
Pathway analysis revealed the involvement of pathways linked to tissue damage in KO mice and pathways involved in tissue repair in WT mice.

WT INF vs WT CT
Vasoconstriction
Development of cardiovascular system
Vasculogenesis
Development of blood vessel
Development of epithelial tissue

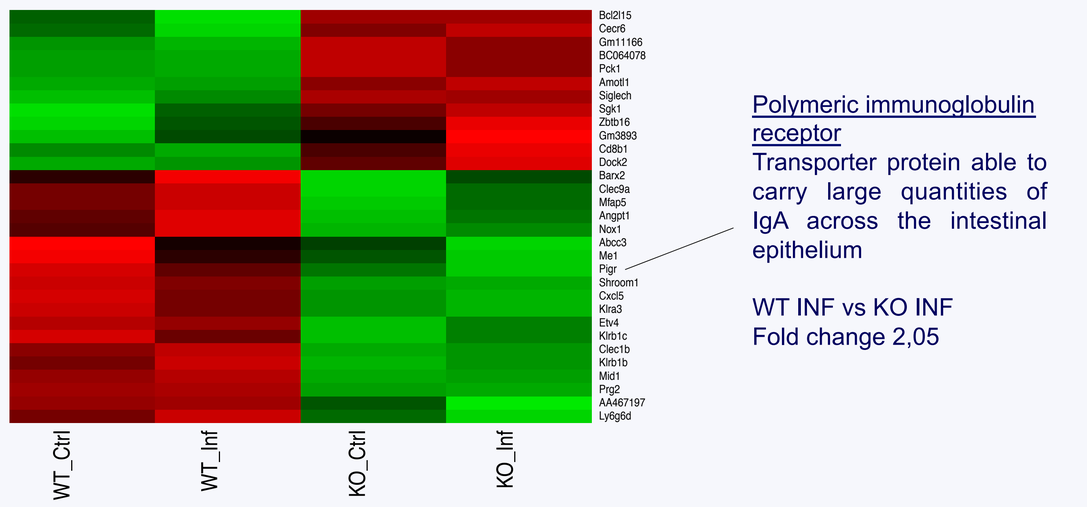
KO INF vs KO CT
Apoptosis of neurons
Quantity of anion
Apoptosis of brain cells
Damage of kidney
Blood pressure

Table 1. Top 5 of the most impacted and increased functions as predicted by IPA in the comparisons WT infected versus WT control and KO infected versus KO control

A heatmap was created with 140 genes that are responsive to infection in either genotype, but that are differentially expressed between WT and KO mice upon infection. A second heatmap provides an overview of 31 differentially expressed genes between WT control versus KO control mice, that differentially respond upon infection in WT versus KO mice.



Heatmap 1. Heatmap of genes that differentially respond upon infection in WT infected mice versus KO infected mice



Heatmap 2. Heatmap of genes that are differentially expressed between WT control versus KO control mice and that differentially respond upon infection in WT infected versus KO infected mice

Conclusions

There is a very dinstinct gene expression pattern in the small intestine of *G. muris* infected C57Bl/6 WT mice versus IL-17RA KO mice, reflected by 287 differentially expressed genes. In IL-17RA KO mice that fail to mount a protective immune response against the parasite, there is evidence for ongoing tissue damage 3 weeks post-infection, instead of tissue repair.

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