Modulation of immune responses to *Clostridium difficile* by peroxisome proliferator-activated receptor γ and miRNA-146b

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Background: *Clostridium difficile* is typically a harmless anaerobic bacterium but has recently re- emerged as a pathogen that can cause nosocomial diarrhea, colitis and death. It grows in the intestine of individuals whose microflora has been altered. Many countries have reported an increase in incidence of *C. difficile*-associated disease (CDAD) over the last years. Current treatments for CDAD do not restore the normal microflora and have not been effective in clostridial clearance, but further prolong *C. difficile* shedding. Hence, the discovery of novel therapeutic approaches that effectively control CDAD is important.
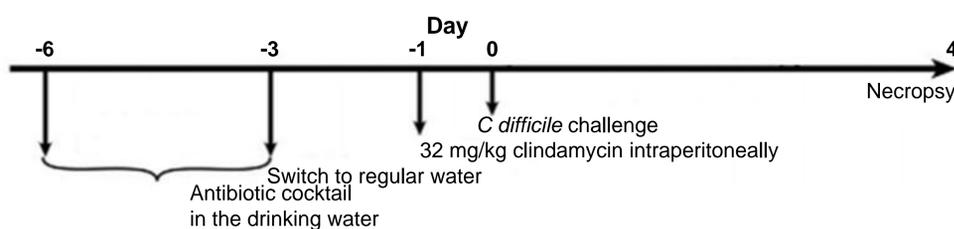

Figure 1. The mouse model of *C. difficile* infection. Mice were treated with an antibiotic cocktail (4.2 mg/kg colistin, 3.5 mg/kg gentamicin, 21.5 mg/kg metronidazole and 4.5 mg/Kg vancomycin) for 3 days in the drinking water followed by a single intraperitoneal clindamyin injection (32 mg/kg). Mice were infected with 10^5-10^7 cfu of *C. difficile* strain VPI 10463. Colons were collected at day 4 post-infection and were examined for colonic microscopic lesions, as well as differential gene and miRNA expression.
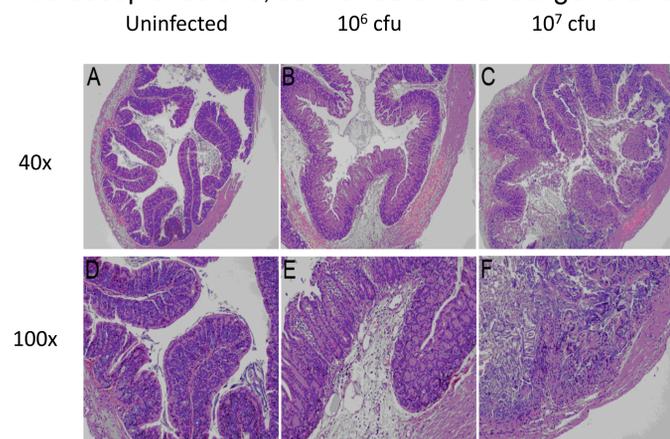

Figure 2. *C. difficile* infection increases colonic microscopic lesions. Significant increase in epithelial erosion, leukocytic infiltration and mucosal thickness as well as more severe inflammatory lesions correlated with increasing infectious doses of *C. difficile*. In addition, microscopic examination revealed extensive areas of necrosis of the mucosa and submucosal edema.

Figure 3. RNA-sequencing analyses revealed an increased expression of miRNA-146b, miRNA-1940 and miRNA-1298 in colons of *C. difficile*-infected mice. Up-regulation of such miRNA was further validated by real-time PCR.
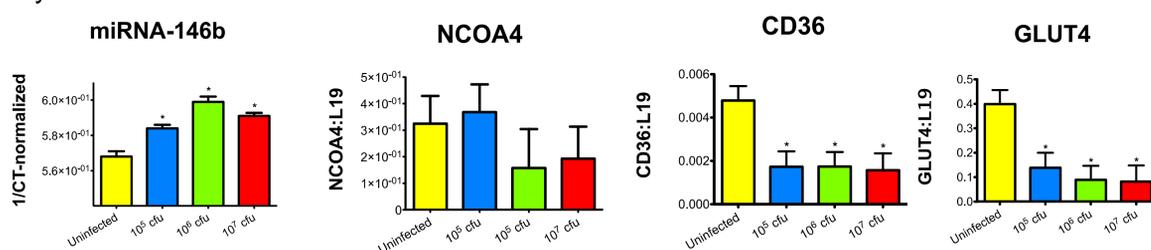

Figure 4. Increased miRNA-146b expression during *C. difficile* infection downregulates PPAR γ activity via NCOA4. Real-time PCR revealed an increased concentration of mmu-miR-146b after *C. difficile* infection. Furthermore, NCOA4, a co-activator of PPAR γ and target of miR- 146b, was down-regulated in colons of infected mice. In line with the suppression of NCOA4 expression via mmu-miRNA146b we found that PPAR γ target genes CD36 and GLUT4 were significantly downregulated in *C. difficile*-infected mice.
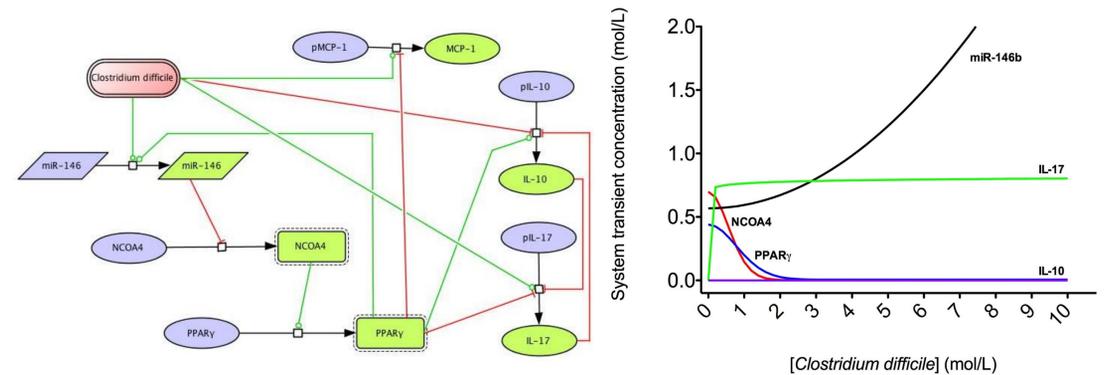

Figure 5. Computational modeling of mucosal immune responses to *Clostridium difficile* infection. Based on the results obtained in the experiments, we are developing an ordinary differential equation-based computational model that shows an aberrant expression of miRNA-146b and IL-17 during *C. difficile* infection, correlating with decreased levels of NCOA4, PPAR γ and IL-10. The network has been constructed using CellDesigner and next imported in SBML into the COmplex PAthway SImulator (COPASI) modeling and simulation software.
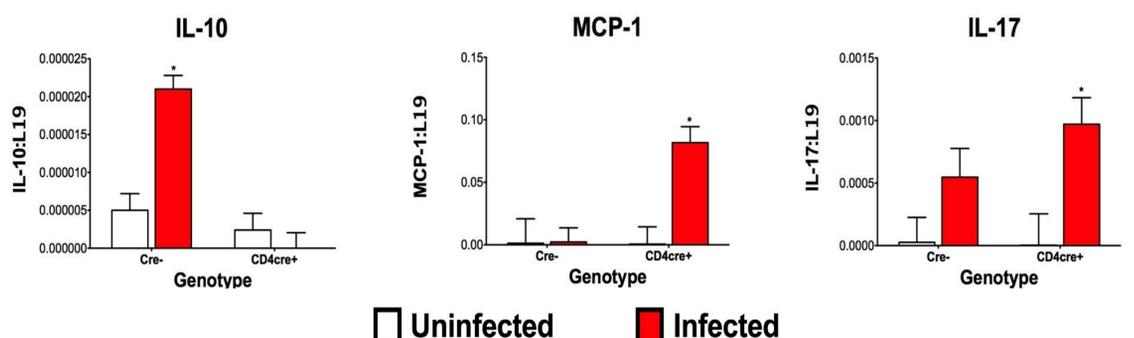

Figure 6. The loss of PPAR γ in T cells resulted in increased colonic inflammation. CD4cre+ mice showed more severe lesions as well as higher expression of pro-inflammatory molecules in the colon.
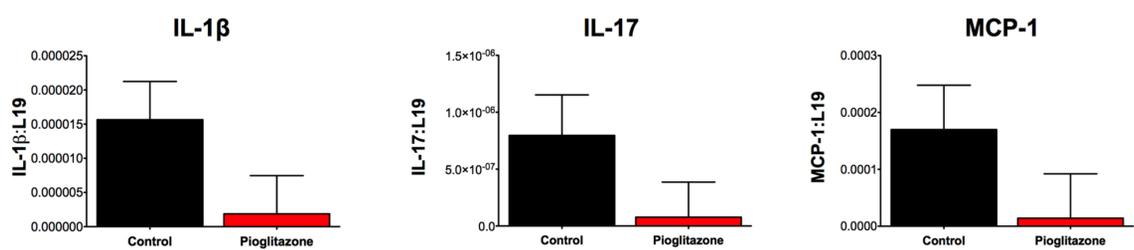

Figure 7. The administration of PPAR γ agonists results in a significant amelioration of CDAD. To assess the possibility of using PPAR γ-based interventions as a therapeutic approach to treat CDAD we treated mice with pioglitazone, thus resulting in a significant amelioration of the disease. Both mucosal thickness and leukocyte infiltration were reduced in colons of treated mice, who also showed decreased expression of pro-inflammatory cytokines.

Conclusions: 1) *C. difficile* increases miRNA-146b expression, which blocks NCOA4, thus reducing PPAR γ activation; 2) The loss of PPAR γ in T cells worsens CDAD; 3) Computational modeling approaches can predict novel molecular target and therapeutics 4) Oral administration of PPAR γ agonists represents a novel broad-based host targeted therapeutic for CDAD.