Th17 responses driven via PPAR γ blockade lead to faster recovery from enteroaggregative *Escherichia coli* infection

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Introduction: Enteroaggregative *Escherichia coli* (EAEC) is the second most common cause of traveler's diarrhea worldwide and possibly the most common cause of diarrheal complaints in the US. Disease pathogenesis is comprised of colonic mucosal adherence, toxin secretion initiating host response, transmigration of neutrophils disrupting lateral tight junctions between epithelial enterocytes, and translocation of the bacteria into the mucosal layer of the intestine exacerbating toxin effects. Mucosal immunity towards EAEC infections is poorly understood. To better characterize immunoregulatory mechanisms underlying EAEC infections nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) was targeted to modulate mucosal immune responses to EAEC. PPARγ is a critical regulator of inflammation suppressing Th1/Th17 and enhancing Treg differentiation with capabilities to modulate macrophage populations. Additionally, to facilitate a systems-level analyses of mucosal immune responses to EAEC, we constructed a computational and mathematical model mimicking host responses to EAEC at the gut mucosa.

Experimental Materials and Methods: Wild type (WT) and conditional knockout mice lacking PPARγ in T cells (CD4-cre+) were fed protein-deficient diets at weaning and challenged with 5x10^{9}cfu EAEC strain JM221. GW9662, a potent PPARγ antagonist, was used to treat mice by oral gavage from day 0 to 7 post infection. Tissue collection occurred on days 5 and 14 post infection.

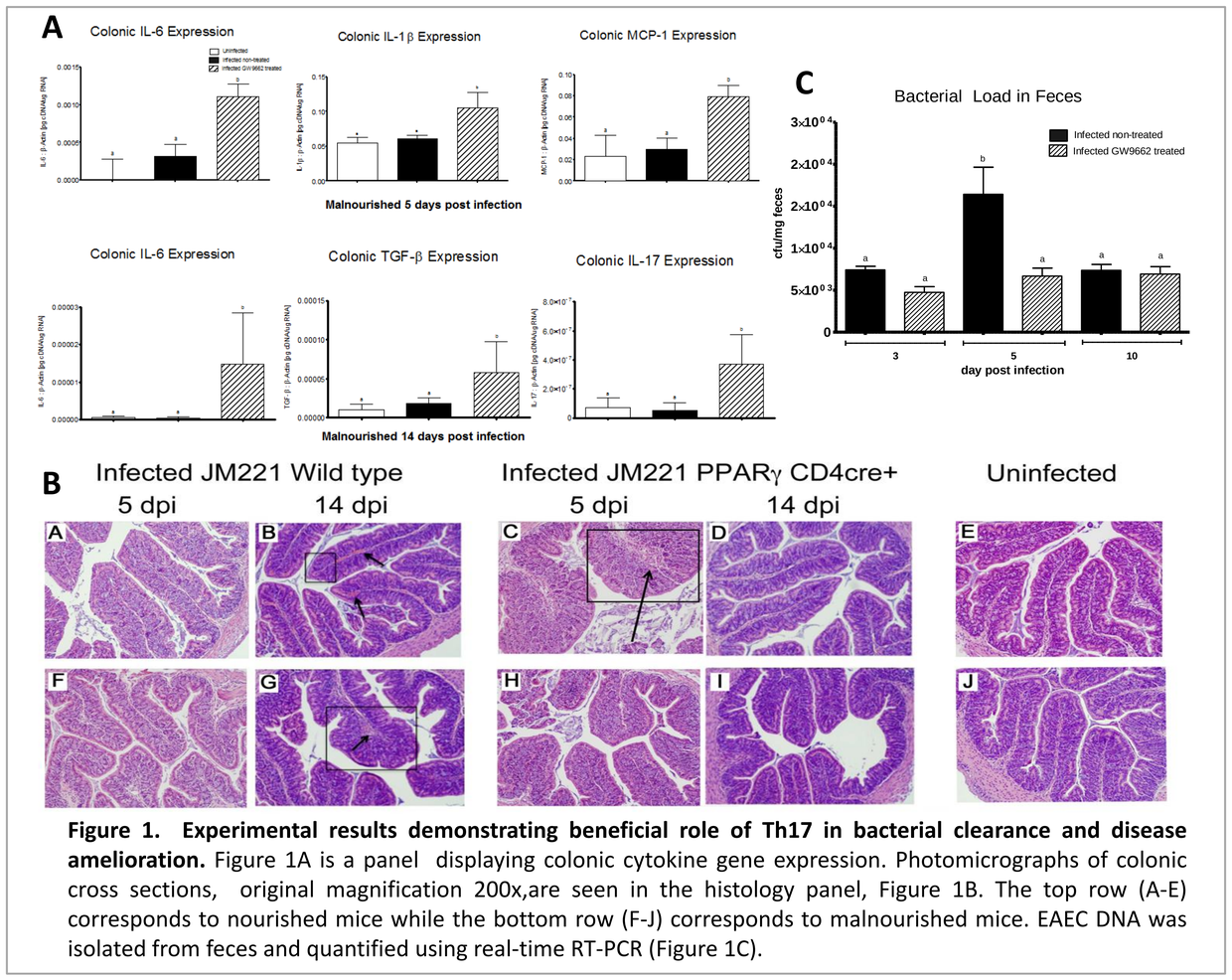

Figure 1. Experimental results demonstrating beneficial role of Th17 in bacterial clearance and disease amelioration. Figure 1A is a panel displaying colonic cytokine gene expression. Photomicrographs of colonic cross sections, original magnification 200x,are seen in the histology panel, Figure 1B. The top row (A-E) corresponds to nourished mice while the bottom row (F-J) corresponds to malnourished mice. EAEC DNA was isolated from feces and quantified using real-time RT-PCR (Figure 1C).

Colonic Gene Expression: Quantitative RT-PCR data reveal that administration of GW9662 for 7 days post infection or the deletion of PPARγ in T cells results in up regulation of pro-inflammatory cytokines including IL-6, MCP-1, and IL-1β when compared to non-treated infected animals early during infection. At 14 days post challenge GW9662 treated mice continued expressing high levels of IL-6, in addition to TGF-β, and IL-17 (Figure 1A).

Histopathological Findings: Histological analysis of colons divulged lower leukocyte infiltration and decreased mucosal thickness 14 days following EAEC infection after pharmacological blockade of PPARγ or deletion of PPARγ in T cells (Figure 1B.A-J).

Bacterial Burden: These findings were accompanied by lower levels of EAEC in fecal shedding for GW9662 treated mice (Figure 1C).

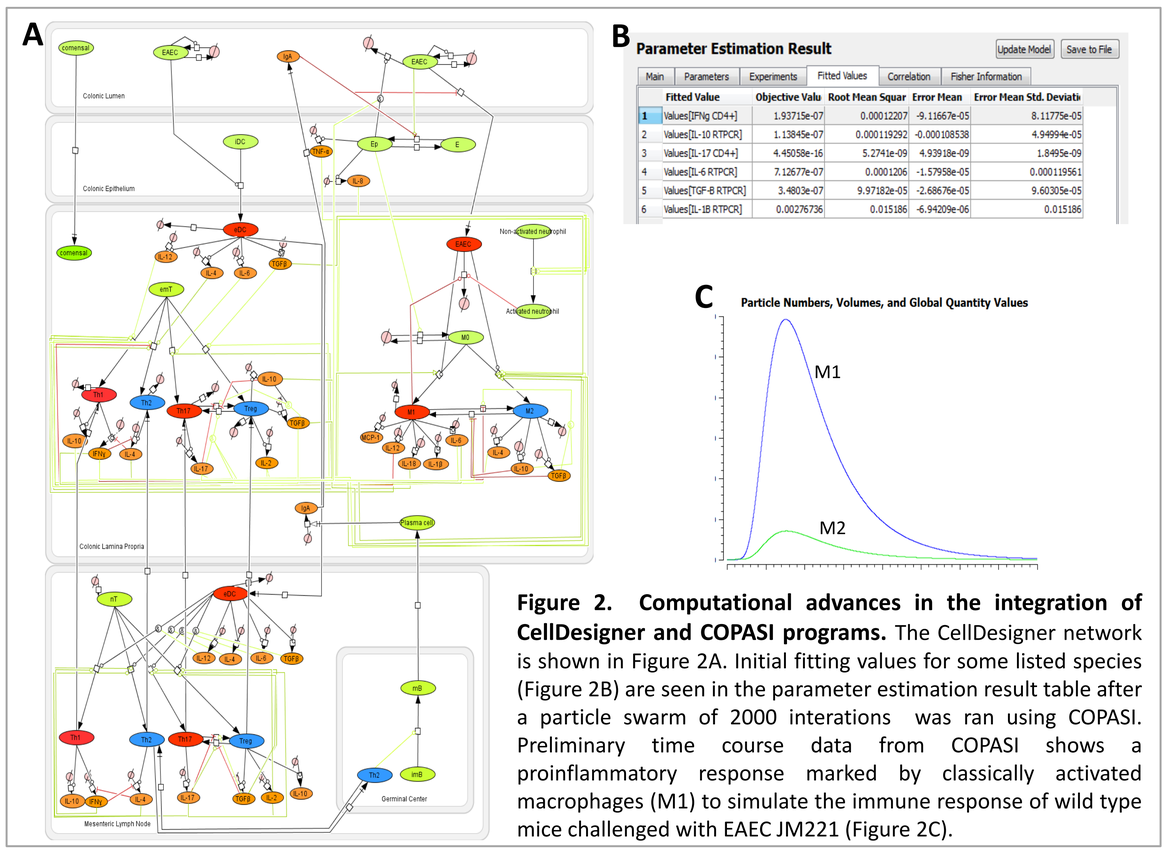

Figure 2. Computational advances in the integration of CellDesigner and COPASI programs. The CellDesigner network is shown in Figure 2A. Initial fitting values for some listed species (Figure 2B) are seen in the parameter estimation result table after a particle swarm of 2000 interations was ran using COPASI. Preliminary time course data from COPASI shows a proinflammatory response marked by classically activated macrophages (M1) to simulate the immune response of wild type mice challenged with EAEC JM221 (Figure 2C).

Computational Efforts: An in depth literature search elucidating host response to EAEC was used to create a CellDesigner network modeling EAEC infection at the mucosal level in the colon. Our multi-compartment network delineates bacteria-epithelial cell interaction providing an initial stimulus triggering a cascade of reactions involving cytokine secretion, neutrophil activation, and macrophage differentiation. Dendritic cells are responsible for antigen presentation and successive differentiation of naïve T cells into their respective phenotype based on fitted environmental conditions (Figure 2A). Our CellDesigner network has been fully integrated with COPASI to function as a deterministic ordinary differential equation (ODE)-based model. Preliminary model calibration efforts demonstrated remarkable fitting to experimental data from infected, untreated, wild type mice (Figure 2B-C).

Conclusions: We have acquired results that provide overwhelming evidence supporting a Th17 effector response toward EAEC challenge when PPAR γ is exogenously blocked by a potent pharmacological antagonist, GW9662. The pro-inflammatory response has unexpected beneficial effects noted by lower bacterial burden and decreased mucosal damage after 14 days of infection when compared to untreated wild type counterparts. Our work presents a fully integrated approach to study host responses to EAEC that provides new insights on the pathogenesis and treatment of enteric pathogens through the assimilation of both computational and experimental standpoints.

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