

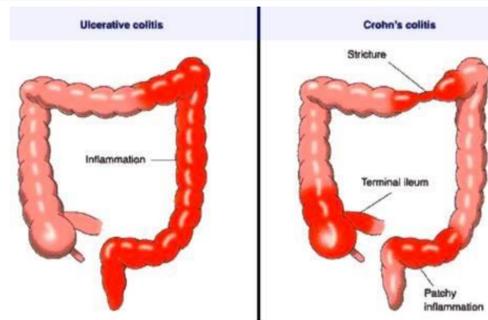
The Role of IFN- γ Induced IL-10R1 Expression in Restitution of Epithelial Barrier Function during Intestinal Inflammation

Nathan A. Blaseg, Heather Walk, Brittany Jenkins, Margaret Lehmann, Douglas Kominsky
Montana State University, Department of Microbiology and Immunology, Bozeman MT 59715

Abstract

Inflammatory bowel diseases (IBD) are debilitating diseases of unknown origin, but a combination of genetic and environmental factors are thought to be involved in disease pathology. Ongoing inflammatory responses are paralleled by significant alterations of epithelial cellular responses, including changes in barrier function and cytokine response. Preliminary studies indicate that epithelial IL-10 signaling plays a critical role in barrier function and tissue restitution. Importantly, during ongoing inflammation IFN- γ mediates the upregulation of IL-10R1 expression. Further, studies in a murine colitis model demonstrate that loss of epithelial IL-10R1 dramatically worsens inflammation and disease outcomes *in vivo*. Based on these preliminary studies, we hypothesize that epithelial IL-10R1 expression is crucial to tissue homeostasis and IFN- γ -induced upregulation of IL-10R1 primes the tissue for pro-resolving IL-10 signaling. To define these principles, two specific aims are being pursued. The first aim is to define the molecular mechanisms of epithelial IL-10-dependent maintenance of barrier function. Here we are focusing on the examination of apical junction proteins and the impact of IL-10 signaling on expression, protein level, and localization of these targets using a host of molecular biology techniques. The second aim is to further investigate the role that differentiation defects may play with regards to the barrier function of epithelial cells lacking IL-10R1. To this end we are investigating expression and protein level of factors critical to intestinal epithelia differentiation *in vitro*, as well as using staining techniques on tissues derived from colitis mouse models. A better understanding of these principles may lead to improved treatment for those suffering from IBD, controlling the inflammatory response and ultimately decreasing the severity of disease.

Introduction



- The inflammatory bowel diseases (IBD) include Crohn's disease and ulcerative colitis.
- Cause is unknown, but a combination of genetic and environmental factors are thought to be responsible.
- Estimated 1.4 million cases in the US, and 25% of new cases are pediatric.
- Ongoing inflammatory responses are characterized by shifts in tissue metabolism, which parallel changes in barrier function and cytokine response.

Background

Figure 1: IL-10R1 loss diminishes IEC barrier. Panel B, TEER measurement of shNTC and IL-10R1 KD T84 cells. TEER measurements were monitored daily beginning 1 day post plating (n=6). Panel C, FITC-dextran (3 KD) paracellular flux assay of control vs. IL-10R1 KD cells. Measurements were collected at 30 min increments after application of FITC-dextran to the apical aspect of IEC monolayers (n=1) (*p<0.05, **p<0.01, ***p<0.005).

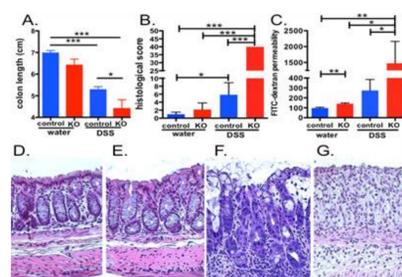
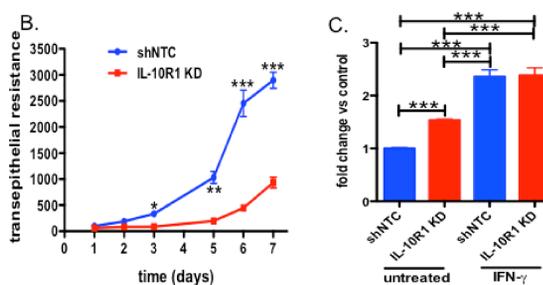
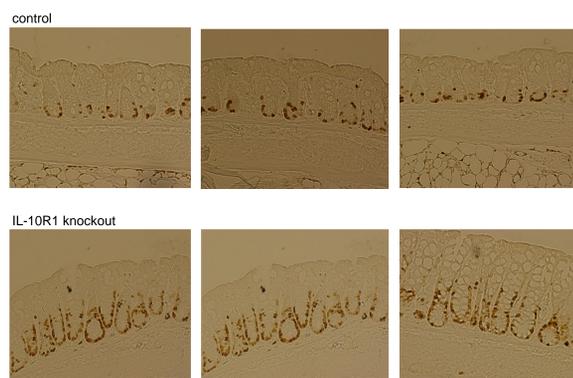
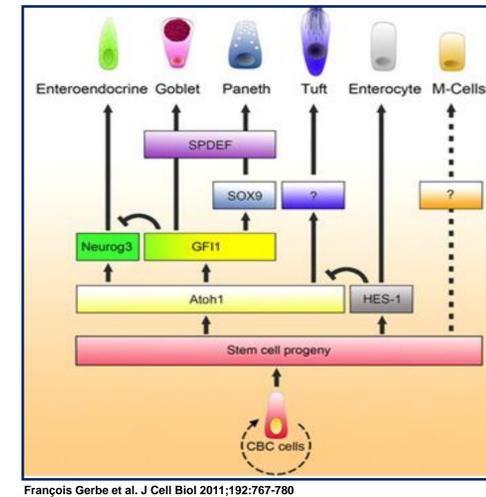
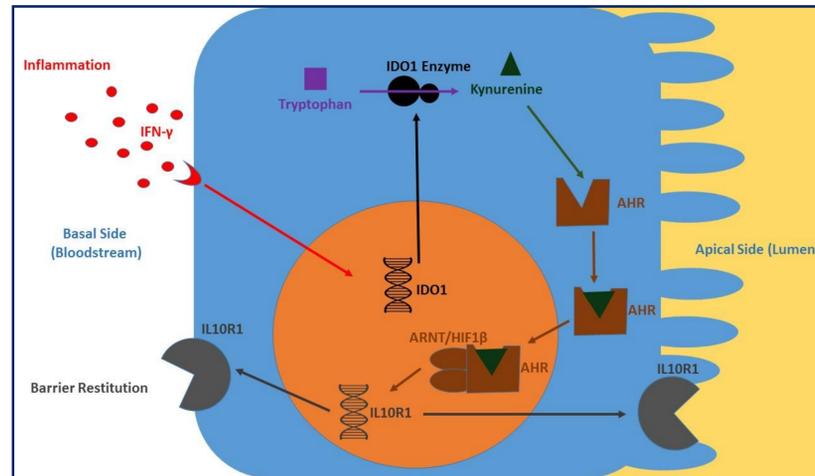


Figure 2: DSS colitis is worsened in IEC IL-10R1 KO mice. 10-12 week old control (cre-, fl/fl) mice and IL-10R1 KO mice were administered 3% DSS for 6 days then allowed to recover for 3 days before harvest. Panel A displays colon length; panel B represents histological score; Panel C, epithelial permeability (n \geq 6, data are expressed as mean \pm SD, *p<0.05, **p<0.01, ***p<0.005). Panel D-G, H&E staining of tissue isolated from control vehicle (D), KO vehicle (E), control DSS (F), and KO DSS (G) animals.

Figure 3: Staining of mouse intestinal tissue revealed cells lacking IL-10R1 showed defects in proliferation. Staining of intestinal tissue harvested from naïve WT mice compared to naïve epithelial IL-10R1 KD mice. Tissue was harvested from 6-12 day-old mice and preserved for histology. Both tissues were stained for cellular differentiation marker Ki-67.

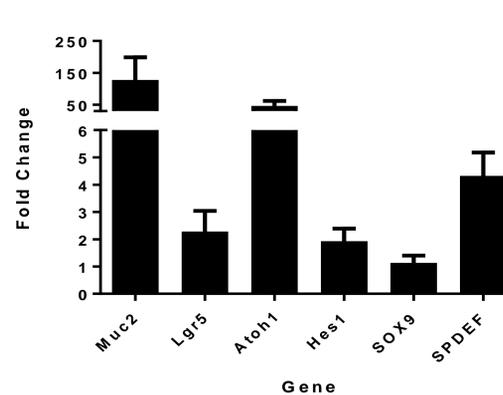


Models



Methods & Results

IL-10R1 KD vs NTC



IL-10R1 KD vs. WT T84

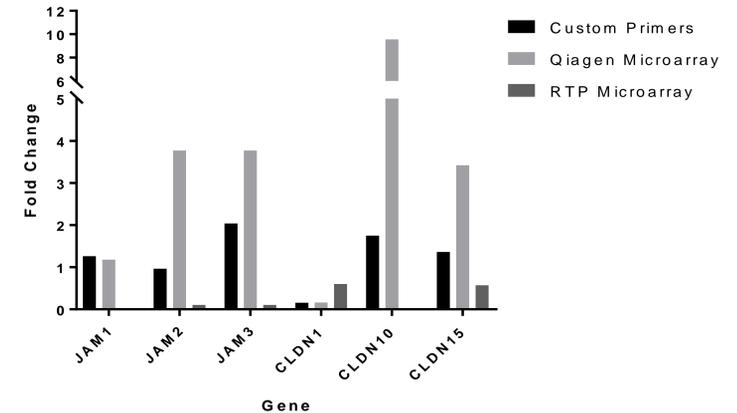


Figure 4: IL-10R1 KD cells show defects in the expression of various differentiation genes when compared to their NTC counterparts. qPCR measurements of IL-10R1 KD T84 human intestinal epithelial cells compared to NTC T84 cells. Cells were harvested after 7 days. RNA was harvested using Trizol reagent and cDNA synthesized. qPCR was then performed using the primers seen (n=7).

Figure 5: IL-10R1 KD cells showed varying levels of expression different tight junction proteins when compared to WT cells. qPCR measurements of IL-10R1KD T84 cells normalized to WT T84 cells. Cells were harvested after 7 days. RNA was harvested using Trizol reagent and cDNA synthesized. qPCR was then performed using microarrays from Qiagen® and Real Time Primers®, as well as custom designed primers. This graph displays junctional adhesion molecules 1, 2, and 3, claudins 1, 10, and 15. These genes were selected for further study after reviewing the microarray results.

Conclusion & Future Research

Our results thus far have helped to further validate our working model and provide us with more potential avenues for research into the expression and function of IL-10R1. Tight junction protein transcript levels have been sufficiently investigated. Junctional adhesion molecules (JAMs) 1-3, as well as Claudins 1, 10, and 15 will be targets of further study. Claudins 10 and 15, both leaky claudins, are of particular interest. Next, protein level will be determined through western blot, and protein localization within the cells will be investigated through the use of immunofluorescence. These studies will hopefully help determine which proteins are essential for the restitution of barrier following inflammation. The differentiation defects seen in epithelial cells lacking IL-10R1 will be further investigated using western blot and immunofluorescence, as well as staining of tissue harvested from mice. Another model which will be used for further examination of this differentiation defect will be the use of *ex vivo* human intestinal organoids. These will be used to better simulate a relevant biological system, and they will provide another potential tool to look at the expression of these various proteins which affect differentiation. These results thus far provide us with important techniques and more specific targets for future research. This continued research will lead to a better understanding of the mechanisms behind barrier restitution during IBD and ultimately contribute to the development of treatments which help to decrease the severity of disease.

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