Novel *Escherichia coli* Derived Therapeutic Immunotherapies Reduce Innate Immune Responses to Protect Muc2 deficient mice from Spontaneous Colitis

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Abstract

Ulcerative Colitis (UC) is a form of inflammatory bowel disease (IBD) in which the intestinal mucosal barrier, including the mucus layer, is compromised -leading to ulceration. Mice that lack the major mucin found in the intestinal mucus, mucin 2 (Muc2), develop spontaneous colitis that mimics UC. QBECO, an immunomodulator derived from an inactivated strain of *Escherichia coli* that holds potential as a novel immunotherapeutic agent for UC by restoring normal innate immune function, was tested in Muc-2 deficient (Muc2 -/-) mice. QBECO administered subcutaneously every 2nd day for 30 days reduced spontaneous colitis in the Muc2 -/- mice. Specifically, QBECO treatment markedly improved the overall histological score, reduced T cell infiltration and decreased neutrophil numbers in the colonic tissues. These observations were accompanied with a reduction in pro-inflammatory mediators IL-17A in the colon and keratinocyte-derived chemokine (KC) in serum. QBECO treatment did not impact regulatory T cell marker (FoxP3) and anti-inflammatory growth factor (TGF-β) expressions in affected tissues. Additionally, QBECO treated mice attenuated levels of the antimicrobial lectins, RegIII-β and RegIII-γ, which favorably affected the gut microbiome by limiting the growth of gamma-proteobacteria and increasing the probiotic lactobacilli. These data demonstrate QBECO treatment ameliorates spontaneous colitis in aged *Muc2 -/-* mice. Together, these findings may have broader implications for our understanding of IBD pathology and aid in the development of novel immunotherapeutic focused on reconstituting normal immune function in the context of IBD.

Background

- Ulcerative colitis is a form of inflammatory bowel disease thought to occur when mucosal barrier is damaged or defective
- Therapeutic approach has been limited and not fully effective
- Mice lacking the mucus protein mucin 2 (Muc2) develop spontaneous colitis that mimics ulcerative colitis
- QBECO is a microbe-based investigational therapeutic (Qu Biologics Inc.) that is an immunomodulator derived from in activated *E. coli*

Hypothesis

QBECO ameliorates spontaneous colitis in Muc2 -/- mice by selectively dampening the innate immune response

Methods

- Three months old *Muc2 -/-* mice (when colitis is established) were subcutaneously injected with placebo or QBECO every other day for 30 days
- Tissues were collected for histology, immunostaining and gene expression analysis
- Blood was collected for cytokine analysis

QBECO treated *Muc2-/-* mice showed reduced colonic tissue damage

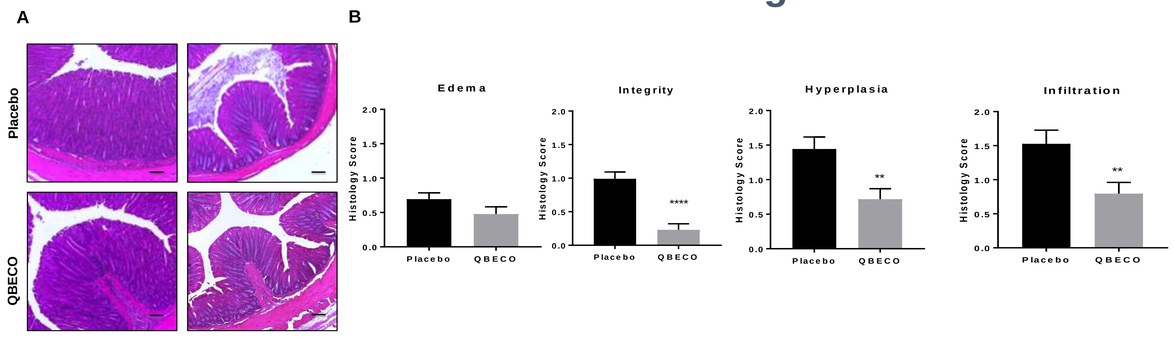

Figure 1: QBECO treatment amelioration of intestinal inflammation in *Muc2 -/-* mice. Muc2 -/- mice were intraperitoneally injected with placebo or QBECO every other day for 30 days and tissues were collected for (A) histological and (B) histology grading. n > 14, mean ± SEM, * p<0.05

QBECO reduced neutrophils infiltration and neutrophils related cytokines in *Muc2 -/-* mice

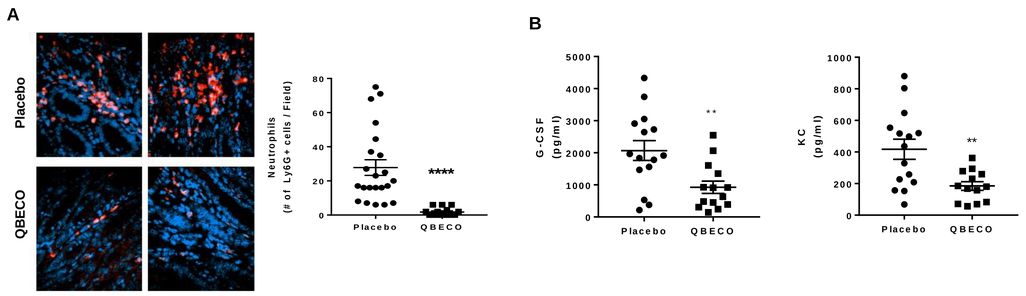

Figure 2: Reduction of neutrophils and neutrophils related cytokines in QBECO treated *Muc2 -/-* mice. (A) Ly6G+ve neutrophils were immunostained (red cells) and enumerated within the distal colon tissues of placebo and QBECO treat *Muc2-/-* mice. (B) G-CSF and KC (neutrophils proliferation and recruitment cytokine) were measured in the serum. QBECO (n=10); placebo (n=10) mice. Student's t test. **p<0.001.

QBECO decreased CD3+ T cell infiltration and transcription of the pro-inflammatory cytokine IL-17A

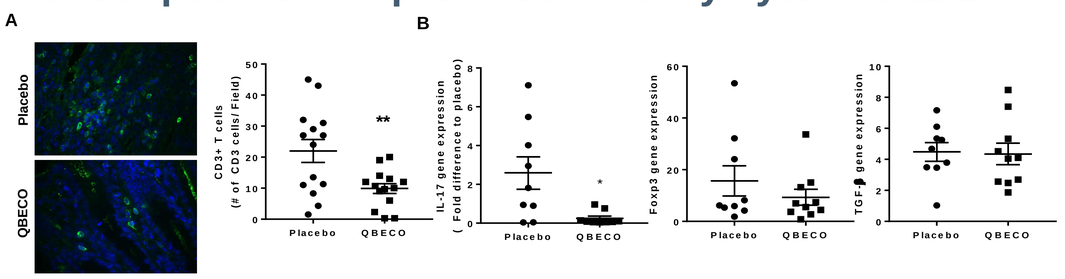

Figure 3: Reduction in CD3+ve T cell numbers and pro-inflammatory cytokine gene transcription in SSI treated Muc2-/- mice: A) CD3+ve T cells were immunostained (green cells) and enumerated within the distal colon tissues of placebo and SSI treat Muc2-/- mice in at least 10 high power fields (x 630) per mouse. (B) Gene transcript levels of pro-inflammatory cytokines IL-17A , Foxp3 and TGF-β. QBECO (n=10); placebo (n=9) mice, Student's t test *p<0.05, ** p<0.01.

QBECO treatment reduced RegIIIβ expression and modulates the gut microbiome

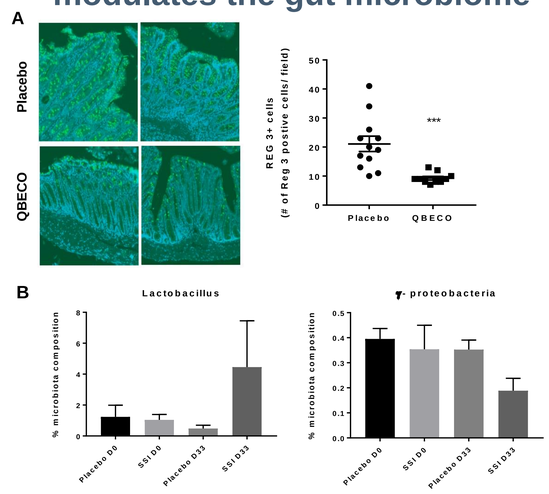

Figure 4: SSI treatment modulates expression of the antimicrobial lectin RegIII-β and shapes the composition of gut microbiota: A) RegIII-**β** cells were immunostained (green cells) and enumerated within the distal colon tissues of placebo and SSI treat Muc2-/- mice B) 16S ribosomal RNA analysis of stool commensal bacteria before and after QBECO treatment. SSI (n=10), placebo (n=9) mice, * p<0.05

Conclusion

QBECO, derived from inactivated *E. coli,* beneficially modulate the spontaneous colitis developed in *Muc2 -/-* mice by:

- Ameliorating intestinal pathology
- Decreasing neutrophils numbers and CD3 positive T cells
- Modulating RegIII-β, with a reduction in γ-proteobacteria and an increase in lactobacilli

This study highlights the potential of using bacterial-derived products for innate immune stimulation as a therapeutic for ulcerative colitis

References

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