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Modulatory Effects of IFN-γ and IL-22 on Inflammatory Signaling and Cellular Responses in Intestinal Epithelial Cells

By

Asia Johnson

Under the Direction of Timothy L. Denning, Ph.D.

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

In the Institute of Biomedical Sciences

Georgia State University

2024

Abstract

Inflammatory bowel disease (IBD) continues to affect millions worldwide, with an increasing prevalence that highlights the urgent need for deeper understanding of its underlying immune mechanisms. The cytokine interactions, especially those mediated by cells from the TH1 and TH17 lymphocyte subsets, are crucial in orchestrating the immune landscape of IBD. TH1 cells are well known for producing TNF-α and IFNγ, which have been extensively studied for their roles in conjunction with each other within the context of IBD (Fish, 1999). TH17 cells secrete IL-22 and IL-17, with existing studies primarily focusing on IL-22's interaction with IL-17 rather than its interplay with other cytokines such as IFNγ. Our study focuses on the co-stimulatory effects of IL-22 and IFNγ using organoids derived from mouse small intestines to model epithelial interactions. We found that IFNγ interferes with the capacity of IL-22 to up-regulate antimicrobial peptides, which is essential in mucosal defense. Additionally, higher concentrations of IL-22 enhance IFNγ's ability to stimulate TNF-α gene expression and CXCL10 protein production, indicating a dose-dependent relationship. This co-stimulation also led to an increased rate of cell death, influenced partly by TNF-α. These findings suggest that IL-22, typically seen as an anti-inflammatory agent, can assume a pro-inflammatory role when combined with IFNγ, complicating its effects on epithelial cells. This study highlights the need to consider specific cytokine interactions in developing more effective IBD treatments.

INDEX WORDS: IFNγ, IL-22, TNFa, CXCL10, IBD

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2024

Modulatory Effects of IFN-γ and IL-22 on Inflammatory Signaling and Cellular Responses in Intestinal Epithelial Cells

By

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May 2024

DEDICATION

I dedicate this dissertation to those that keep me on this mortal plane.

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I would like to express my sincere gratitude to all the staff members whose daily efforts facilitate our research environment. Special thanks to the administrative and security teams for their indispensable support. You made many late nights in the lab possible without being featured in a campus alert. A huge thanks to T'Keyah Johnson for keeping me updated on deadlines and the next steps.

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TABLE OF CONTENTS

DEDICATION .... **v**

ACKNOWLEDGEMENTS .... **vi**

LIST OF TABLES .... **x**

LIST OF FIGURES .... **xi**

LIST OF ABBREVIATIONS .... **xiii**

1. Introduction **1**

1.1 Inflammatory Bowel Disease 1

1.2 Factors of IBD 1

1.3 Epithelial Barrier Function and Components 3

1.4 Epithelial Barrier and IBD Susceptibility Genes 4

1.5 Epithelial Barrier and the Microbiota in IBD 4

1.6 Epithelial Barrier and Immune Response Relationship 5

1.7 IL-22 6

1.8 IL-22 in IBD 6

1.9 IL-22 at the Epithelial Barrier 7

1.10 IFNy 7

1.11 IFN-γ in IBD 8

1.12 IFN-γ at the Epithelial Barrier 9

1.13 IBD Therapeutics and Future Directions 9

1.14 CXCR3 and its Ligands 9

1.15 Inhibition of IL-23/IL12 10

2. Modulatory Effects of IFN-γ and IL-22 on Inflammatory Signaling and Cellular Responses in Intestinal Epithelial Cells 12

2.1 Introduction 12

2.2 Materials and Methods 12

2.2.1 Animal Procurement and Housing 12

2.2.2 Crypt Isolation and Organoid Culture Maintenance 13

2.2.3 Mode-K Cell Culture 14

2.2.4 Propidium Iodide/ Hoechst Live Dead Staining 14

2.2.5 ELISA 14

2.2.6 Immunofluorescence Microscopy 15

2.2.7 RNA Isolation and cDNA Synthesis 15

2.2.8 Quantitative PCR 16

2.2.9 Scratch Assay 16

2.2.9 qPCR Primers 17

2.2.10 Statistical Analysis 17

2.2.11 Use of AI Language Model 17

2.3 Results 18

2.3.1 IFNy Dampens IL-22 Up Regulation of REG3b and REG3y 18

2.3.2 IL-22 Amplifies IFNy-induced TNFa Gene Expression and Protein Induction in a Dose-dependent Manner 18

2.3.3 Higher Concentrations of IL-22 Amplify the CXCL10 Protein Induction Capabilities of IFNy 19

2.3.4 Barrier Integrity 19

2.3.5 Il-22 and IFNy Synergize, Enhancing Cell Death in Organoids 20

2.3.6 Anti-TNFa Partially Ameliorates Cytokine-induced Cell Death 21

2.3.7 IL-22 and IFNy Co-stimulation Slows Wound Closure 22

2.3.8 IL-22 and IFNy Interfere with the Other's Signaling Pathway 22

2.3.8 Mode-k Cells Do Not Reflect the Results Observed in Organoids 23

2.3.9 TNFα Does Not Inhibit IL-22-induced AMP Expression 23

4. References 38

5. VITAE 55

LIST OF TABLES

Table 1. QPCR Primer Sequences. 17

LIST OF FIGURES

Figure 1. The Etiology of IBD is Multifactorial. 3

Figure 2. STAT Signaling Pathways. 8

Figure 3. IL-22 and IFNy at the Epithelial Barrier. 11

Figure 4. Organoid Gene Expression in Response to IFNy and IL-22 Stimulation. 24

Figure 4.1. IL-22 and IFNy Co-stimulatory Effects on AMP Expression. 25

Figure 4.2. IL-22 and IFNy Co-stimulatory Effects on TNFa Gene Expression and Protein Induction. 25

Figure 4.3. IFNy and IL-22 Co-stimulation on CXCL10 Protein Levels in Organoid Culture Media. 26

Figure 4.4. PI/H Image of Organoids Treated with IFNy at Different Concentrations for 48 Hours. 27

Figure 4.5. PI/H Images of Organoids Treated with IL-22 at Different Concentrations for 48 Hours. 28

Figure 4.6. Cytokine Stimulation on Organoid Cell Viability. 29

Figure 4.7. PI/H Images of Organoids After 48 Hours of Stimulation. 30

Figure 4.8. IFNy and IL-22 Co-stimulation on Cell Viability of Organoids. 31

Figure 4.9. Anti-TNFa on Organoid Cell Viability. 31

Figure 4.10. Digital Phase Contrast Images of Scratch Assay on Mode-k Cells. 32

Figure 4.11. IL-22 and IFNy Co-stimulation in Wound Healing. 32

Figure 4.12. Immunofluorescence of Phospho-STATS in Mode-K Cells. 33

Figure 4.13. Mode-k Cells PSTATs in Response to Cytokine Stimulation. 33

Figure 4.14. IL-22 and IFNy Stimulatione Effects on Mode-k Cells. 34

Figure 4.15. IL-22 and TNFa Co-stimulation of Organoids. 34

LIST OF ABBREVIATIONS

No.	Abbreviation	Definition
1.	AIEC	Adherent-invasive Escherichia coli
2.	AMP	Antimicrobial Peptide
3.	ATG16L1	Autophagy Related 16 Like 1
4.	CARD9	Caspase Recruitment Domain-containing Protein 9
5.	CD	Crohn's Disease
6.	CDH1	Cadherin 1
7.	CLEC7A	C-type Lectin Domain Family 7 Member A
8.	CXCL	C-X-C Motif Chemokine Ligand
9.	CXCR	C-X-C Motif Chemokine Receptor
10.	DCs	Dendritic Cells
11.	DSS	Dextran Sodium Sulfate
12.	FDA	Food and Drug Administration
13.	GAS	IFN-γ Activated Site
14.	GI	Gastrointestinal
15.	HSV-1	Herpes Simplex Virus Type 1

16.	IBD	Inflammatory Bowel Disease
17.	IECs	Intestinal Epithelial Cells
18.	IFN-y	Interferon Gamma
19.	IL	Interleukin
20.	IL-22R1	IL-22 Receptor Subunit Alpha 1
21.	ILC	Innate Lymphoid Cells
22.	ISGs	Interferon-stimulated Genes
23.	JAK	Janus Kinase
24.	JAK-STAT	Janus Kinase-Signal Transducer and Activator of Transcription
25.	NK cells	Natural Killer Cells
26.	NOD	Nucleotide-binding Oligomerization Domain-containing Protein
27.	NOD-like	NOD-like
28.	PI/H	Propidium Iodide/Hoechst
29.	PSTAT	Phosphorylated Signal Transducer and Activator of Transcription
30.	PTPN2	Protein Tyrosine Phosphatase Non-Receptor Type 2

31.	QPCR	Quantitative Polymerase Chain Reaction
32.	REG3	Regenerating Islet-Derived Protein 3
33.	SI	Small Intestine
34.	SNP	Single Nucleotide Polymorphism
35.	SOCS1	Suppressor of Cytokine Signaling 1
36.	STAT	Signal Transducer and Activator of Transcription
37.	TGF-β	Transforming Growth Factor Beta
38.	TNF-α	Tumor Necrosis Factor Alpha
39.	Tregs	Regulatory T Cells
40.	UC	Ulcerative Colitis

1. Introduction

1.1 Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC), both of which are marked by persistent inflammation within the gastrointestinal (GI) tract (McDowell, 2023). CD manifests anywhere along the GI tract but predominantly targets the small intestine, whereas UC specifically affects the large intestine. The etiology of IBD has been attributed to a complex interplay of factors, including microbiota dysbiosis, genetic predispositions, immune system dysregulation, and environmental factors (McDowell, 2023; Hu, 2024).

The prevalence of IBD is notably higher in Western societies, with newly industrialized regions experiencing a rise in incidence rates (Ng et al., 2017). Lifestyle differences, such as diet, are suspected to drive this trend (Chiba, 2019). In the United States, the impact of IBD on society is profound, with annual direct and indirect costs per patient ranging between $7,000 and $41,000 (Kahn-Boesel, 2023; Burisch, 2023). Considering a prevalence rate surpassing 0.7% of the population, IBD poses a significant public health challenge (Lewis, 2023).

1.2 Factors of IBD

Environmental factors contributing to the risk of developing IBD include obesity, stress, antibiotic use, diet, and exercise. Obesity shares a positive correlation with the increased risk of IBD, potentially elucidating the escalating prevalence of IBD in the USA alongside rising obesity rates (Carreras-Torres, 2022; NIH, 2021). Conversely, exercise is negatively associated with the risk of IBD. Individuals with IBD face a

heightened risk of colorectal cancer and psychological comorbidities, including anxiety and depression (Shah, 2022; Barberio, 2021).

The disease's complex pathology is reflected in identifying over 240 gene loci associated with IBD (Liu, 2023). A range of susceptibility genes, such as NOD2, ATG16L1, CARD9, IL-23R, and CLEC7A, play a significant role in barrier defense and are instrumental in immune pathways that ensure a balanced relationship with the microbiota (Cohen, 2021; Lang, 2019; Santana, 2019; Zhang, 2015; Jarmakiewicz-Czaja, 2022).

The microbiota's involvement in IBD is scrutinized as a causative agent and a focal point for therapeutic intervention. Dysbiosis is a common observation in IBD cases, prompting numerous studies to delve into the microbiota's impact on IBD pathology and how various factors influence this dynamic. Alpha diversity decreases in the microbiota of patients with IBD, and fluctuations in composition occur more often compared to controls (Qui2022, Abdel-Rahman, 2023). Reducing commensal bacteria and increasing pathogenic bacteria further drive an environment prone to excess inflammation. (Qui, 2022). Fecal matter transplantation is among the innovative treatments under investigation for IBD (Kedia, 2022). The relationship between diet, the microbiota, and human health is an area of research that is of great interest. Research indicates Westernized diets are more prone to dysbiosis, and diets rich in fiber are conducive to microbiomes with more diversity and a higher ratio of commensal bacteria (Martinez, 2021)

At the core of IBD lies a sustained pro-inflammatory response. Pinpointing the origins of this response and devising strategies for its management are needed. The

epithelial barrier is the critical juncture where the principal contributing factors of IBD—host genetics, the microbiota, environmental triggers, and the host immune system—converge. A deeper understanding of the intricate interplay among these elements is crucial for developing efficacious treatment modalities for IBD. Subsequent sections will further explore contributing factors.

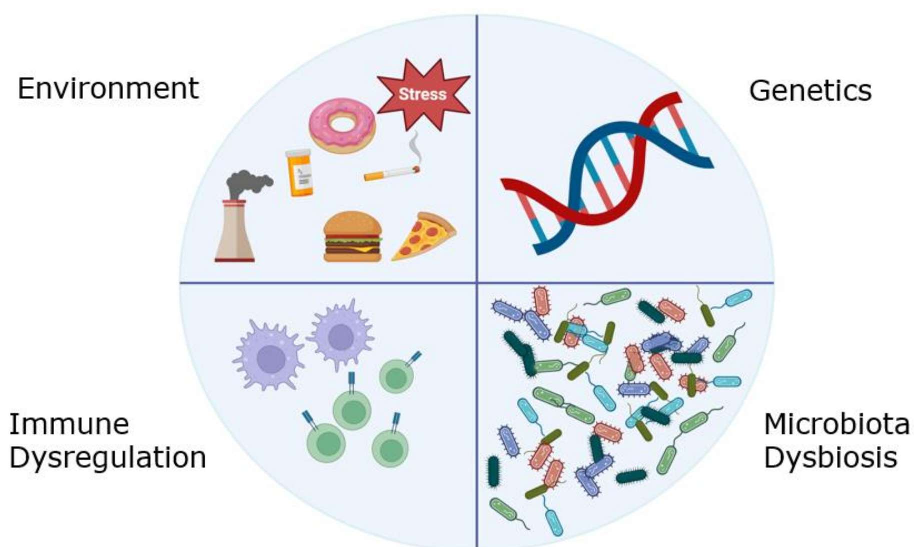

Figure 1. The Etiology of IBD is Multifactorial.
Environment, genetic predisposition, immune dysregulation and microbiota dysbiosis all play a part in pathogenesis.

1.3 Epithelial Barrier Function and Components

The intestinal epithelium absorbs nutrients and water, eliminates waste, and acts as a barrier between the intestinal lumen and the immune system beneath it (Odenwald, 2017). This barrier's selective permeability is maintained by a layer of varied epithelial cells linked by tight junctions, including claudins, occludins, and junction adhesion molecules, ensuring compartmentalization (Otani, 2020). The surface facing the lumen is shielded by a mucus layer, predominantly produced by goblet cells situated within the epithelial layer. These cells are essential for generating mucins that contribute to the mucus layer's consistency and thickness, a feature that varies between the small and

large intestines and is influenced by the gut microbiota (Gierynska, 2022). Located beneath the epithelial cells, on the side facing the host, is the lamina propria—a connective tissue enriched with diverse immune cells (Buckley, 2018). The integrity of the epithelial and mucus layers is vital to prevent detrimental interactions between the microbiota, other luminal substances, and the immune system.

1.4 Epithelial Barrier and IBD Susceptibility Genes

IBD susceptibility genes impact barrier integrity through various mechanisms. E-cadherin, a critical adherens junction protein, is essential for maintaining cell-cell adhesion and tight junction formation (Barret, 2009). Specific haplotypes of CDH1 are associated with UC and are linked to increased cytoplasmic localization of E-cadherin, leading to junctional disruption, a common feature in inflammation (Muise, 2009; McCole, 2014). The SNP rs2542151 in protein tyrosine phosphatase non-receptor type 2 (PTPN2) is strongly associated with IBD, where the gene product, T-cell protein tyrosine phosphatase (TCPTP), is elevated in patients with active CD. Knockdown of PTPN2 results in a barrier defect driven by IFN-γ (Scharl, 2009). Alleles of Muc3A produce proteins with altered conformations that may be more susceptible to degradation by bacterial proteases, weakening the barrier (Kyo, 2001; Van den Steen, 1998). Additionally, CARD15/NOD2 mutations correlate with increased intestinal permeability in CD patients (Buhner, 2006).

1.5 Epithelial Barrier and the Microbiota in IBD

The epithelial barrier shapes the microbiota composition through mechanisms such as autophagy, peptide secretion, dispersion of microRNAs, and provision of alternative energy sources (Kwon, 2019; Salzman, 2010; Liu, 2016; Goto, 2016). The

microbiota, in turn, sustains barrier function by providing microbial metabolites that enhance nutrient and drug metabolism, activate NOD-like and Toll-like receptors, and produce antimicrobial substances (Cheng, 2008; Ey, 2009; Ghosh, 2021)

A symbiotic microbial environment is crucial for the barrier's function and modulation of the immune response. In IBD, microbiota composition and displacement changes are expected (Okumura, 2018). Both experimental setups and clinical samples have detected bacteria in the typically germ-free inner mucus layer, contributing to an inappropriate immune response and exacerbating inflammation (Linares, 2021; Okumura, 2018). Pathogenic shifts include displacing commensal bacteria like Roseburia intestinalis by pathogenic species such as Bacteroides fragilis (Vich Vila, 2018). Adherent-invasive Escherichia coli (AIEC) is particularly implicated in IBD due to its ability to adhere to epithelial cells and evade autophagy (Pamela, 2018). In CEABAC10 mice, a Western diet promotes AIEC colonization, which is associated with mucus layer thinning, increased intestinal permeability, and elevated TNFα (Martinez, 2014). The role of dysbiosis in IBD as either a consequence or contributor to altered barrier function varies and is case-dependent, yet invariably leads to immune activation and a pro-inflammatory environment.

1.6 Epithelial Barrier and Immune Response Relationship

The epithelial barrier significantly modulates the immune response. Intestinal epithelial cells (IECs) secrete critical factors like transforming growth factor (TGF)-β and retinoic acid, which are both important in the development of Foxp3+ regulatory t cells (Tregs) (Coombes, 2007). The presentation of microbial antigens by IECs is key in

activating Tregs. Tregs must mature for immune tolerance (Atarashi, 2013; Arpaia, 2013; Smith, 2013; Soderholm, 2019).

In its interaction with the barrier, the immune system triggers differentiation, proliferation, and inflammatory responses. Receptors on the epithelial cells' apical and basolateral sides participate in various activities. Releasing cytokines and chemokines from immune cells that bind to these receptors on epithelial cells amplifies the immune response (citation).

Understanding IBD necessitates a comprehensive grasp of the immune system's role, which is characterized by an intricate network of cytokines and chemokines. Numerous cytokines and chemokines engage in complex interactions involving a variety of cells and multiple immune factors, implicating them in IBD.

1.7 IL-22

Interleukin-22 (IL-22), belonging to the IL-10 cytokine family, plays a vital role in maintaining and repairing epithelial barriers, which is essential for protecting mucosal surfaces against microbial invasion and promoting tissue repair (Kier, 2020). Produced by various immune cells, including Th17, Th22, dendritic cells (DCs), and Group 3 innate lymphoid cells (ILC3), IL-22 acts on epithelial cells through the IL-22R1 receptor, predominantly triggering the JAK-STAT signaling pathway. This action results in the phosphorylation, dimerization, and nuclear translocation of STAT3. IL-22 activates the PSTAT1 and PSTAT5 pathways to a lesser extent (Horn, 2024).

1.8 IL-22 in IBD

In healthy individuals, IL-22 is typically absent from the large intestine but is constitutively present in the small intestine (Mizoguchi, 2018). Researchers have

observed elevated levels of IL-22 in samples from both UC and CD patients. CD IL-22-related pathology is largely part of a TH1/ TH17 response. In UC, the dominant relevant response is a TH2 response, and there are lower levels of IL22 compared to a TH1/TH17 response (Mizoguchi, 2018; Abraham, 2009).

1.9 IL-22 at the Epithelial Barrier

IL-22 is crucial in maintaining the integrity of the epithelial barrier by stimulating mucin and antimicrobial peptide (AMP) production. This cytokine not only induces mucin production but also promotes the proliferation of goblet cells (Kier, 2020). IL-22 is vital for tissue repair following acute injury. The absence of IL-22 impairs tissue regeneration (Aparicio-Domingo, 2015), and IL-22-deficient mice exhibit increased susceptibility to Citrobacter rodentium infection, highlighting its protective role in host defense (Zheng, 2008). While IL-22 contributes to maintaining intestinal homeostasis and modulating the immune environment through its various functions, the transition to the role of another critical cytokine, IFN-γ, highlights a different aspect of immune regulation.

1.10 IFNy

IFN-γ is the only type 2 interferon and is produced by TH1, CD8+ T cells, NK cells, and ILC1 (Murray, 2002; Conlon, 2021) Binding of IFN-γ to its receptor activates the JAK1 and JAK2 pathways, resulting in the phosphorylation and dimerization of STAT1. These homodimers then translocate to the nucleus to bind to IFN-γ activated site (GAS) elements on IFN-stimulated genes (ISGs), initiating transcription (Platanias, 2005). Interferon-gamma is crucial for defending against intracellular bacterial and viral pathogens. Deficiencies in IFN-γ significantly increase susceptibility to infections by organisms such as Salmonella and HSV-1 (Perez-Toledo, 2020; Kim, 2022).

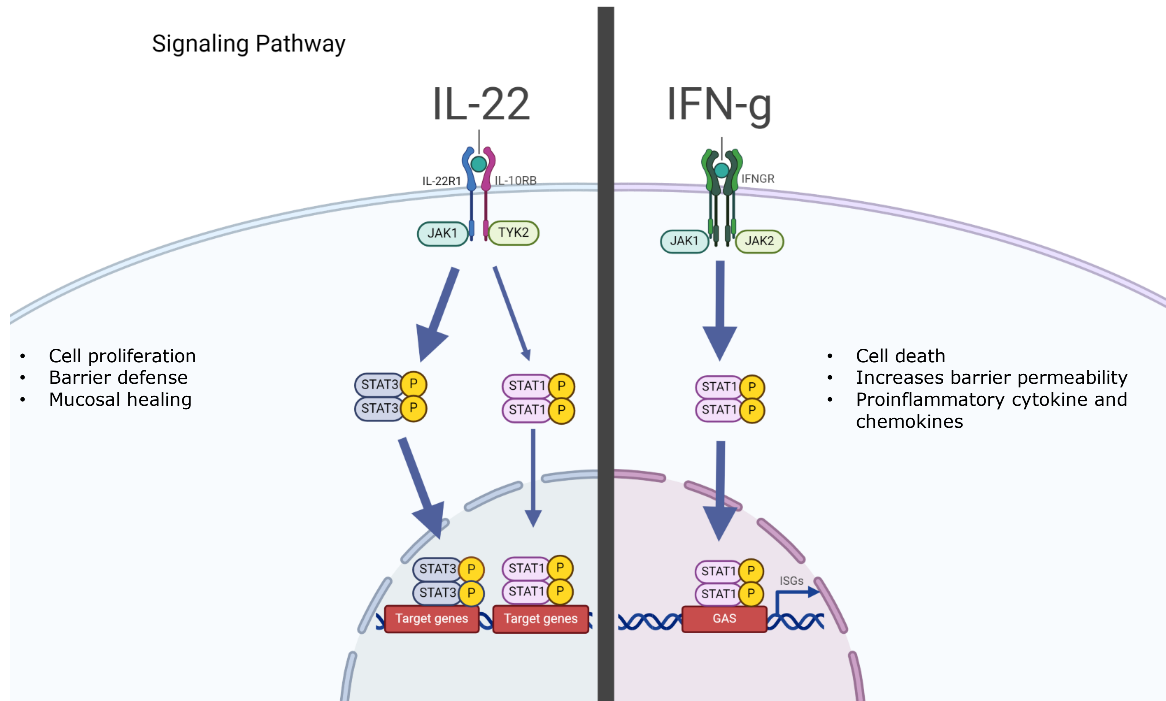

Figure 2. STAT Signaling Pathways.
IL-22 primarily signals through STAT3 and IFNy primarily signals through STAT1.

1.11 IFN-γ in IBD

IFN-γ often plays a detrimental role in inflammatory bowel disease (IBD). The polymorphism IFNG rs1861494 is associated with increased IFN-γ secretion and more severe IBD symptoms (Gonsky, 2014). Transcriptomic studies have found elevated IFNg gene expression in UC patients (Gao, 2022).

IFN-γ contributes negatively in several colitis models, enhancing cell apoptosis/necroptosis and inflammatory cytokine production. In the DSS colitis model, IFN-γ knockout mice do not develop colitis, unlike their wild-type counterparts (Ito, 2006). Cigarette smoke-induced colitis shows that IFN-γ knockout mice experience less TNF-α elevation, reduced weight loss, and better colon length preservation than heterozygous mice (Nava, 2010). Additionally, IFN-γ has been shown to promote bacterial translocation across the intestinal epithelium (Clark, 2005) and works synergistically with TNF-α in many models in driving inflammation.

1.12 IFN-γ at the Epithelial Barrier

The influence of IFN-γ on the epithelial barrier is significantly affected by other immune mediators. Its pro-inflammatory effects increase in the presence of TNF-α, whereas SOCS1 inhibits its signaling pathways (Larkin, 2013). IFN-γ disrupts barrier integrity by downregulating junction proteins and promoting bacterial translocation (Clark, 2005; Han, 2019). IFN-γ stimulation induces CXCL9, CXCL10, and CXCL11 secretion from epithelial cells, attracting cytotoxic T cells and NK cells that exacerbate inflammation (Suzuki, 2007; Dwinell, 2001; Kulkarni, 2017).

1.13 IBD Therapeutics and Future Directions

TNFa is the most targeted factor for therapeutics in IBD treatment, with the majority of IBD treatments being biologics against TNFa; as many as a third of IBD patients are primary non-responders to TNFa and about half of those that initially respond to treatment end up secondary non-responders (Kumar, 2024). Despite identifying TNFa as a prominent contributor to IBD pathology, targeting TNFa alone is not an adequate treatment.

1.14 CXCR3 and its Ligands

Targeting CXCR3 and its ligand CXCL10 is a promising approach in chemokine therapy for IBD (Trivedi, 2018). High CXCR3 expression on activated T-cells has been observed in IBD patient tissue biopsies, with increased CXCL10 potentially leading to epithelial cell death. The chemokines CXCL9, CXCL10, and CXCL11 are all elevated in preclinical models of UC, with CXCR3 ligands attracting CD4+CXCR3+ T cells to the epithelial barrier, exacerbating inflammation (Suzuki, 2007; Dwinell, 2001; Kulkarni, 2017). While these chemokines are often grouped, they play different roles in the

immune response. CXCL10 only binds to CXCR3 and is noted as particularly important in its involvement in autoimmunity(Christen, 2003).

1.15 Inhibition of IL-23/IL12

The inhibition of IL-23 and IL-12 is a targeted strategy for treating inflammatory bowel disease (IBD), with several FDA-approved biologics designed to suppress the effects of these cytokines. However, the clinical outcomes of targeting both or either cytokine have shown variable efficacy. This variability underscores the importance of understanding the complex downstream interactions mediated by IL-12 and IL-23 in IBD pathogenesis.

Upon stimulation by environmental factors and microbial antigens, dendritic cells, and macrophages produce IL-12 and IL-23, essential in differentiating naïve T-cells into TH1 and TH17 cells. TH1 cells primarily produce TNF-α and IFN-γ, while TH17 cells secrete IL-22 and IL-17 (Roda, 2020). While extensive research has focused on the impact of TNF-α and IFN-γ on epithelial cells, and some studies have explored the effects of IL-22 and IL-17, the simultaneous influence of IL-22 and IFN-γ on intestinal epithelial cells remains unexplored (Woznicki, 2021; Pavlidis, 2022).

Given the critical roles these cytokines play within the IL-12/IL-23 pathway, particularly at the epithelial barrier, it is essential to investigate their combined effects on intestinal epithelial cells. Such studies could provide valuable insights into their synergistic or antagonistic interactions, potentially leading to more effective therapeutic strategies for IBD.

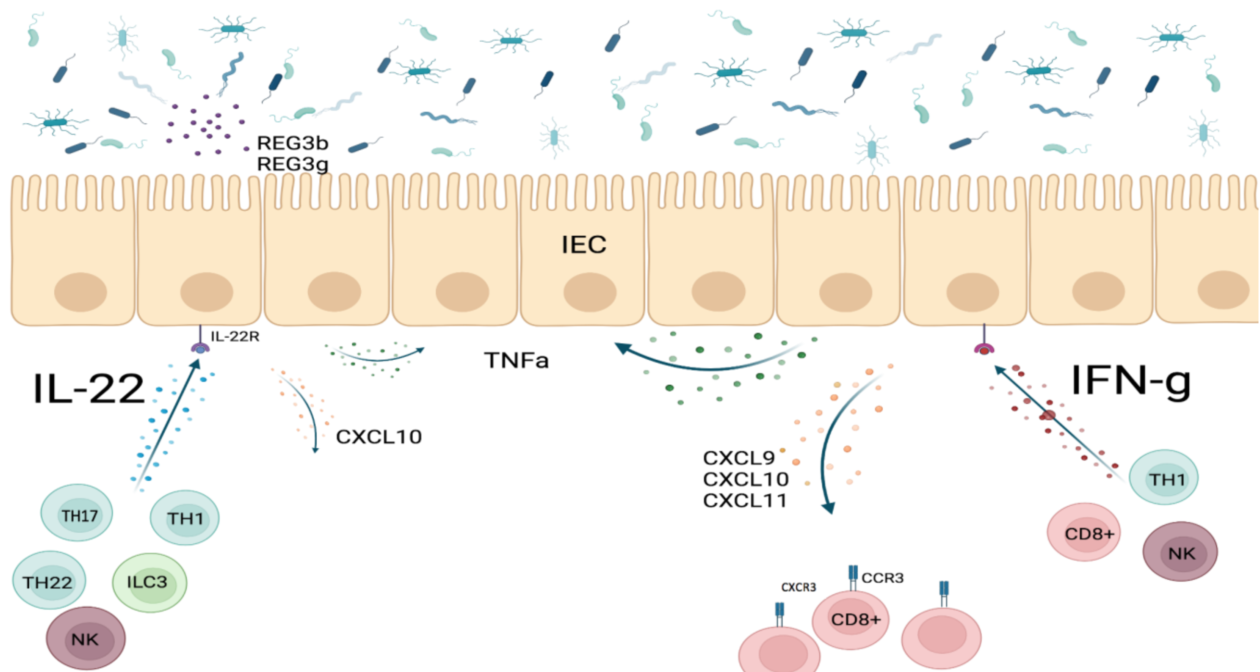

Figure 3. IL-22 and IFNy at the Epithelial Barrier.
IL-22 stimulation induces AMP production and to a lesser degree CXCL10 and TNFa. IFNy Stimulation up-regulates CXCL9, CXCL10, CXCL11.

2. Modulatory Effects of IFN-γ and IL-22 on Inflammatory Signaling and Cellular Responses in Intestinal Epithelial Cells

2.1 Introduction

Understanding IBD necessitates a comprehensive grasp of the immune system's role, which is characterized by an intricate network of cytokines and chemokines. The cytokine interactions, especially those mediated by cells from the TH1 and TH17 lymphocyte subsets, are crucial in orchestrating the immune landscape of IBD. TH1 cells are well known for producing TNF-α and IFNγ, which have been extensively studied for their roles in conjunction with each other within the context of IBD (Fish, 1999). TH17 cells secrete IL-22 and IL-17, with existing studies primarily focusing on IL-22's interaction with IL-17 rather than its interplay with other cytokines such as IFNγ. Using mouse small intestine derived organoids we explored the effects IL-22 and IFNy co-stimulation on epithelial cells.

2.2 Materials and Methods

2.2.1 Animal Procurement and Housing

Male and female B6 mice aged 8-12 weeks were obtained from Jackson Laboratories. Animals were housed under controlled conditions with a 12-hour light/dark cycle, temperature maintained at 22 ± 2°C. They were given access to standard laboratory chow and water ad libitum throughout the study period.

All animal experiments were conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care

and Use Committee (IACUC) of the host institution. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2.2 Crypt Isolation and Organoid Culture Maintenance

Intestinal crypt isolation was performed using B6 mice. The intestine was removed and flushed with Dulbecco's Phosphate Buffered Saline (GIBCO) containing 0.1% Bovine Serum Albumin (DPBS 0.1% BSA (Sigma Aldrich)). The intestines were then sectioned into 3 mm pieces and washed with DPBS 0.1% BSA. The tissue segments were incubated at 37°C with Gentle Cell Dissociation Reagent (Stemcell Technologies) for 5 minutes while shaking at 100 rpm. Subsequently, the tissue was subjected to vigorous manual shaking for 15 seconds and then briefly vortexed. The supernatant was discarded, and fresh 37°C Gentle Cell Dissociation Reagent was added before placing the tissue back in the shaker for an additional 15 minutes. This was followed by a 15–20-minute incubation at room temperature without shaking. The tissue was then vigorously shaken by hand for 15 seconds, and the supernatant was filtered through a 100 µm cell strainer. DPBS 0.1% BSA was added, and the process was repeated twice more. The final supernatant, containing the isolated crypts, was passed through a 70 µm filter. The crypts were resuspended in a solution of Matrigel (Corning Cat #354248) and Murine IntestiCult (Stemcell Technologies) at a concentration of 60 crypts per 10 µl and seeded into a 96-well plate. Matrigel was diluted to 1x concentration using DMEM (Corning) supplemented with 50ug/mL gentamycin (Gibco).

Organoid cultures were maintained by refreshing the media every two to three days. The cultures were initially passaged on day six following seeding, with

subsequent passages taking place every three to four days thereafter. Experiments were conducted on organoids within three passages from primary isolation to ensure consistency and viability of the samples.

2.2.3 Mode-K Cell Culture

Mode-K cells were retrieved from liquid nitrogen storage and cultured in T75 flasks. The culture media were replenished every 2 to 3 days, and the cells were passaged at 6-day intervals. The Mode-K culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS), 1% HEPES buffer (Gibco), 1% sodium pyruvate (Corning) 0.1% beta-mercaptoethanol (Gibco), and penicillin/streptomycin. For experiments, Mode-K cells were seeded into 96 or 12 well TC treated plates.

2.2.4 Propidium Iodide/ Hoechst Live Dead Staining

Organoids and Mode-K cells were incubated at 37°C with 5% CO_2 in culture media supplemented with 5 µg/mL PureBlu Hoechst (Bio-Rad) and 5 µg/mL Propidium Iodide, for a duration of 45 minutes. Following incubation, the culture media was discarded, and the cells were washed with pre-warmed PBS. The plates were then analyzed using a Cytation 5 imaging reader (Agilent Technologies). A 7x7 area scan was conducted for fluorescence detection at the following settings: Excitation at 535/20 nm and Emission at 617/20 nm for Propidium Iodide, and Excitation at 361/20 nm and Emission at 486/20 nm for Hoechst staining.

2.2.5 ELISA

ELISAs for CXCL10 and TNF-alpha were performed using Duo-set kits from R&D Systems following the manufacturer's guidelines. The assays included multiple wash

steps performed on a designated machine with a bottom wash followed by three additional washes. The blocking step involved reagent diluents at 5% concentration. Absorbance was measured using a Cytation 5 imaging reader (Agilent Technologies) at 450 nm, with 570 nm serving as the reference wavelength.

2.2.6 Immunofluorescence Microscopy

The culture medium was discarded, and the cells were washed with PBS. Cells were then fixed and permeabilized in 100% ice-cold methanol at -20°C for 4-5 minutes. After the removal of methanol, cells were washed again with PBS and subsequently blocked with 5% goat serum for 1 hour at room temperature with gentle shaking. Following another PBS wash, primary antibodies (Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145 and Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb #9167, Cell Signaling) were diluted at a ratio of 1:200 and applied to the cells, which were then left to incubate overnight at 4°C.

The next day, the primary antibody solution was removed, and cells were washed with PBS. Secondary antibodies (Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 555, Invitrogen) were diluted between 1:500 and 1:1000 and added to the wells. Cells were incubated for 1 hour with gentle shaking in the dark. Afterward, the secondary antibody was discarded, and cells were stained with DAPI in PBS for 15 minutes with shaking at room temperature. A final wash with PBS was performed before acquiring images on a Cytation 5 imaging system. Analysis was performed using Gen5 Software by Biotek (Agilent).

2.2.7 RNA Isolation and cDNA Synthesis

Total RNA was extracted from tissue samples using Qiagen's RNeasy Mini Spin Columns, incorporating an on-column DNase digestion to eliminate genomic DNA contamination. cDNA was synthesized using the SuperScript IV Reverse Transcriptase kit from Thermo Fisher Scientific, with a modified protocol that included an extended incubation at 50°C to improve transcript yield.

2.2.8 Quantitative PCR

Gene expression levels were quantified by qPCR using iTaq Universal SYBR Green Supermix (Bio-Rad). Specific primers for each gene, detailed in Table [Table Name], were used. Reactions were performed on a QuantStudio 5 Real-Time PCR System according to the standard SYBR Green protocol. All samples were analyzed in duplicate to ensure accuracy and reproducibility.

2.2.9 Scratch Assay

The scratch assay was performed on mode-k cells that were cultured to near or full confluency in 96-well plates. A sterile 1000 µl pipette tip connected to a vacuum system was used to create a uniform scratch across the cell monolayer. Subsequent to the scratch, cells were maintained in their growth medium under standard culture conditions. Brightfield microscopy images were captured at 0 and 36 hours post-scratch using a Cytation 5 Imaging Reader. Image analysis for quantifying wound closure was conducted using ImageJ software, employing the Scratch Wound Assay Macro developed by the MRI Group. This analysis provided quantitative data on cell migration and wound healing over time.

2.2.9 qPCR Primers

Primers	5’ Forward 3’	5’Reverse3’
GAPDH	CGACTTCAACAGCAACTCCCACTCTTCC	TGGGTGGTCCAGGGTTTCTTACTCTT
REG3y	TTCCTGTCCTCCATGATCAAAA	CATCCACCTCTGTTGGGTTCA
REG3b	ATGCTGCTCTCCTGCCTGATG	CTAATGCGTGCGGAGGGTATATTC
CXCL10	GGATGGCTGTCCTAGCTCTG	TGAGCTAGGGAGGACAAGGA
TNFa	CGATCACCCCGAAGTTCAGTA	CAGGCGGTGCCTATGTCTC

Table 1. QPCR Primer Sequences.

2.2.10 Statistical Analysis

All statistical analysis were performed using Graphpad Prism 10.1.2. Ordinary One-Way ANOVA followed by Tukey’s Multi-Comparisons tests. T-test were used for groups of two. ns=not significant.

2.2.11 Use of AI Language Model

ChatGPT, an AI language model developed by OpenAI, was utilized during the preparation of this draft. The general workflow was as follows: First, a human writes a draft with all the information. The draft is given to ChatGPT with the prompt: “Please rewrite the following so it flows better. Put the in-text citations into APA format. Do not remove or add any information. In-text citations need to remain with their facts. Please do not use the word “pivotal.” Please correct spelling and grammar mistakes. Avoid introductory clauses”. The author would take the rewritten content and edit what ChatGPT wrote because, invariably, it has failed some of the requests in the prompt. In a second method, ChatGPT would be given the main ideas wanted in a section and asked to write that section. The output was typically exceptionally unfit, but the general structure was useful. So, the author would use the structure to write their content and

then employ method one. The author does not think the process was faster than writing without ai assistance, but the manuscript is probably less painful for others to read.

2.3 Results

REG3b, REG3g, TNFa, and CXCL10, selected for their relevance to inflammatory bowel disease (IBD) and robust expression in small intestine (SI) derived organoids, were investigated in response to stimulation by IFNy and IL-22 (Fig.4).

2.3.1 IFNy Dampens IL-22 Up Regulation of REG3b and REG3y

To explore the co-stimulatory effects of IFNγ and IL-22 on Intestinal epithelial cells, we stimulated small intestine-derived organoids with cytokines for 24 hours and evaluated gene expression by QPCR. After 48 hours of stimulation, we evaluated protein levels in the supernatant by ELISA. IL-22 stimulation increased REG3b and REG3y expression at doses of 1ng/mL and 10ng/mL (Fig4.1.A,B). When 1ng/mL of IFNγ was added to the organoids along with IL-22, a significant reduction in REG3b and REG3y expression was observed (Fig.4.A,B).

2.3.2 IL-22 Amplifies IFNy-induced TNFa Gene Expression and Protein Induction in a Dose-dependent Manner

Organoids co-stimulated with 1ng/mL of IFNγ and 1ng/mL of IL-22 did not express TNFα significantly more than organoids stimulated with IFNγ alone (Fig.4.2A). In contrast, combining 1ng/mL IFNγ with 10ng/mL IL-22 significantly increased TNFα expression (Fig.4.2A). Next we confirmed the QPCR data by ELISA. When 1ng/mL IL-22 was added to the 1ng/mL IFNγ stimulation, TNFα levels showed a significant increase compared to the levels induced by either cytokine alone (Fig.4.2B). Further enhancement of TNFα expression was observed when the concentration of IL-22 was

increased to 10ng/mL while maintaining IFNγ at 1ng/mL. The TNFα levels in this condition were significantly higher than those observed with the lower dose combination of 1ng/mL IL-22 and 1ng/mL IFNγ (Fig.4.2B).

2.3.3 Higher Concentrations of IL-22 Amplify the CXCL10 Protein Induction Capabilities of IFNy

Previous studies have shown IFNy and TNFa co-stimulation of epithelial cells upregulates ISGs including CXCL10 (Oslund, 2014). Having confirmed a significant increase of TNFa in the supernatant of the organoids co-stimulated with IFNy and IL-22, we predicted these conditions would increase concentrations of CXCL10. To test this we employed the same experimental design as when evaluating for TNFa, but used a lower dose of IFNy.

Administration of 0.1ng/mL IFNγ alone did not induce a significant amount of CXCL10 compared to PBS control (Fig.4.3). When 1ng/mL IL-22 was combined with 0.1ng/mL IFNγ, there was a significant enhancement in CXCL10 levels compared to the stimulation with IL-22 alone (Fig.4.3). However, this combination did not result in a statistically significant difference from the CXCL10 levels induced by IFNγ alone. The most notable increase in CXCL10 protein levels occurred when 0.1ng/mL IFNγ was combined with 10ng/mL IL-22 (Fig.4.3). This combination significantly elevated CXCL10 induction above all other tested conditions.

2.3.4 Barrier Integrity

An essential aspect of epithelial barrier defense and IBD pathology is cell death. A breakdown in the barrier allows for the contents of the lumen and the immune system to come into contact, risking elevated immune activation and perpetuation of

inflammation (Odenwald, 2017). IFNγ is known to induce cell death through apoptosis/necroptosis. TNFa has also been studied as a factor capable of inducing cell death in epithelial cells (Woznicki, 2021). Given the results showing an increase in TNFa in the combined co-stimulatory conditions, we were interested in exploring possible effects on cell viability. We employed a propidium iodide and Hoechst staining assay (modified from Bode 2019) to quantitatively measure cell death in mouse intestinal organoids following exposure to varying concentrations of the cytokines over 48 hours.

The percentage of cell death in the organoids demonstrated a dose-dependent increase when stimulated with IFNγ. The cell death rate escalated with higher doses of IFNγ and reached a plateau at 10ng/mL, where it stabilized at approximately 60% mortality (Fig.4.6A). In contrast, organoids stimulated with IL-22 exhibited a different pattern of cell death. The mortality rate increased with escalating doses of IL-22 but reached a plateau at a lower concentration of 5ng/mL, where cell death accounted for about 20% of the population (Fig.4.6B).

2.3.5 Il-22 and IFNy Synergize, Enhancing Cell Death in Organoids

Organoids treated with 0.1ng/mL IFNγ alone caused minimal cell death (Fig.4.8A). Co-stimulation with IL-22 at 1ng/mL and IFNγ at 0.1ng/mL resulted in a significant increase in cell death compared to IFNγ alone (Fig.4.8A). A more pronounced cytotoxic effect was observed when the concentration of IL-22 was increased to 10ng/mL while maintaining IFNγ at 0.1ng/mL. Under these conditions, approximately 50% of the organoid(s) underwent cell death after 48 hours of stimulation (Fig.4.8A).

The same experiment was conducted but with IFNγ at 1ng/mL, with similar results. IFNγ alone induced substantial cell death, which was augmented by co-stimulation with IL-22 (Fig.4.8B). In this experiment, the co-stimulatory conditions exhibited significantly more cell death than the cytokines individually (Fig.4.8B). Additionally, the higher dose of IL-22 resulted in more cell death than the lower dose of IL-22 in the combined conditions (Fig.4.8B).

2.3.6 Anti-TNFa Partially Ameliorates Cytokine-induced Cell Death

Next, we investigated TNFa as a possible contributor to the increase in cell death observed in the co-stimulatory conditions. Organoids were stimulated with 0.1ng/mL of IFNγ and 10ng/mL of IL-22 for 48 hours. One group of organoids was also treated with anti-TNFα. The anti-TNFα-treated group exhibited significantly reduced cell death compared to the group that did not receive anti-TNFα (Fig.4.9A).

In the IL-22 experiments for cell death, at varying doses, after a concentration of 5ng/ml, around 20%, cell death invariably occurs in organoids (Fig.4.6B). We predicted this cytotoxicity to be partially attributed to TNFa, so we mirrored the last experiment. However, we focused on IL-22 10ng/mL stimulated organoids. The group treated with anti-TNFα showed significantly less cell death than the untreated group (Fig.4.9B).

Since we also observed an increase in CXCL10 and previous studies have shown that CXCL10 can induce cell death (Singh, 2009), we explored CXCL10's potential contribution to cell death. We tested responses to combined IL-22 and IFNγ co-stimulation under four different conditions: no inhibitors, anti-TNFα, anti-CXCL10, and a combination of both inhibitors. The introduction of anti-TNFα alone markedly decreased cell death compared to the untreated control (Fig.4.9C). Conversely, anti-

CXCL10, whether alone or combined with anti-TNFα, did not significantly affect cell death (Fig.4.9C).

2.3.7 IL-22 and IFNy Co-stimulation Slows Wound Closure

Further investigating the effects of co-stimulation of IFNγ and IL22 and barrier integrity, we used mode-k cells to perform a scratch/wound healing assay. Previous studies have found that IL-22 generally accelerates the rate of wound closure through proliferation and migration (Avitibile, 2015). IFNγ in wound healing assays promotes closure through migration in certain cell types at specific doses (He, 2017).

In this experiment, mode-k cells were grown to/near confluence, a scratch was made in the monolayer, and after 36 hours, the scratch was assessed for percent closure. Prior to the scratch, the cells were pre-stimulated with cytokines.

IL-22 at higher doses (10ng/mL) accelerated wound closure compared to PBS (Fig.4.11A), while IFNγ slowed wound closure (Fig.4.11B). Co-stimulation with IL-22 and IFNγ impaired wound healing more than IFNγ alone (Fig.4.11B).

2.3.8 IL-22 and IFNy Interfere with the Other's Signaling Pathway

Pickert 2009 linked IL-22 and phosphorylated STAT3 (PSTAT3) to mucosal wound healing, with the absence of STAT3 in IECs significantly impairing healing capabilities. Given the results of the scratch assays, we predicted IL-22 would enhance Phosphorylated STAT3, and co-stimulation with IFNγ would negate IL-22-stimulated elevated PSTAT3 levels. Mode-K cells were stimulated with cytokines for 24 hours and then evaluated for PSTAT3 and PSTAT1 via immunofluorescence.

When Mode-K cells were stimulated with IL-22 at 10ng/mL, the number of PSTAT3-positive cells compared to the baseline and other experimental groups was

pronouncedly increased (Fig.3E) IF Images (Fig.S1A). Co-stimulation of cells with IL-22 at 10ng/mL and IFNγ at 1ng/mL reduced the number of pSTAT3-positive cells, reverting to baseline levels (Fig.3E).

Stimulation of Mode-K cells with IFNγ alone significantly increased the presence of pSTAT1-positive cells (Fig.3F). IL-22 at 10ng/mL was introduced alongside IFNγ, there was a noticeable reduction in pSTAT1-positive cells, although levels remained above baseline (Fig.3F) IF Images (Fig.S1B).

2.3.8 Mode-k Cells Do Not Reflect the Results Observed in Organoids

Mode-k cells were stimulated for 24 hours then gene expression was evaluated by QPCR. Supernatant protein levels were assessed after 48 hours of stimulation by ELISA. Mode-k cells showed no detectable gene expression for REG3b and REG3y (no graph due to complete lack of detection). Mode-k cells showed no basal or stimulus-induced TNFα gene expression above levels comparable to PBS controls (Fig.4.14A). However, CXCL10 is expressed constitutively in Mode-k cells, with IFNγ further upregulating CXCL10 (Fig.4.14B). ELISA confirmed high basal levels of CXCL10 protein (Fig.4.14C). Notably, the co-stimulation of Mode-k cells with IFNγ and IL-22 did not synergistically enhance CXCL10 levels (Fig.4.14C).

2.3.9 TNFα Does Not Inhibit IL-22-induced AMP Expression

Since the data thus far provides a potential role for TNFa in the unexpected effects IL-22 has on epithelial cells, in the presence of IFNy, we wanted to explore co-stimulation of IL-22 and TNFa on organoids.

Organoids from small intestines were stimulated with TNFα and IL-22 for 24 hours, and gene expression was evaluated by QPCR. Unlike IFN-γ, TNFα did not

suppress the up-regulation of AMP gene expression induced by IL-22 (Fig.4.14A,B). Co-stimulation with IL-22 and TNFa significantly enhanced CXCL10 expression compared to other conditions (Fig.4.14C), while TNFα expression in co-stimulated organoids was not distinct from TNFα alone (Fig.4.14D). Apart from REG3y expression, these results are preliminary and warrant further verification.

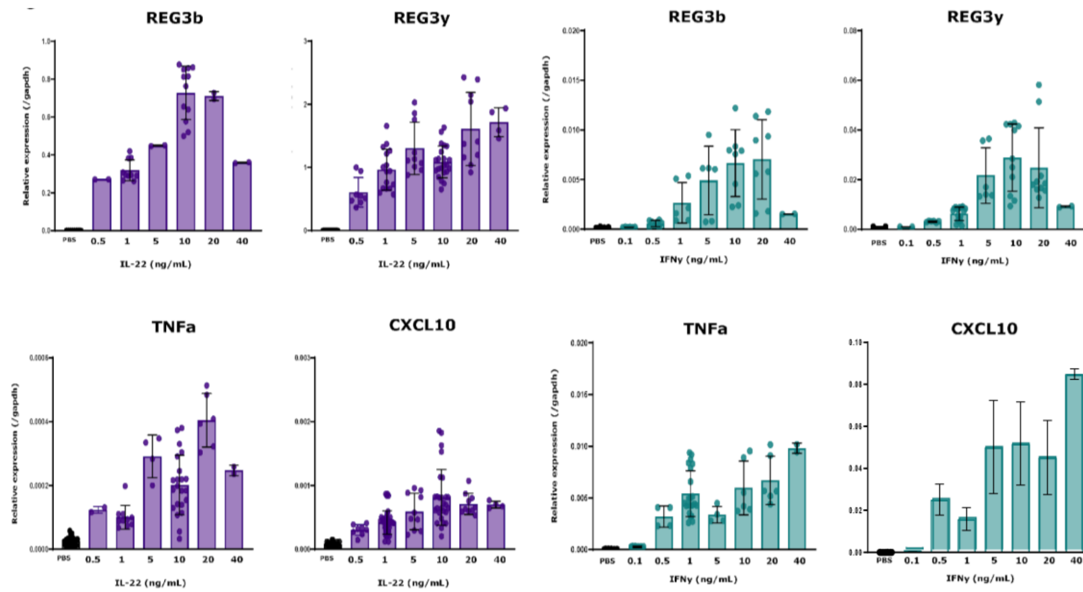

Figure 4. Organoid Gene Expression in Response to IFNy and IL-22 Stimulation.

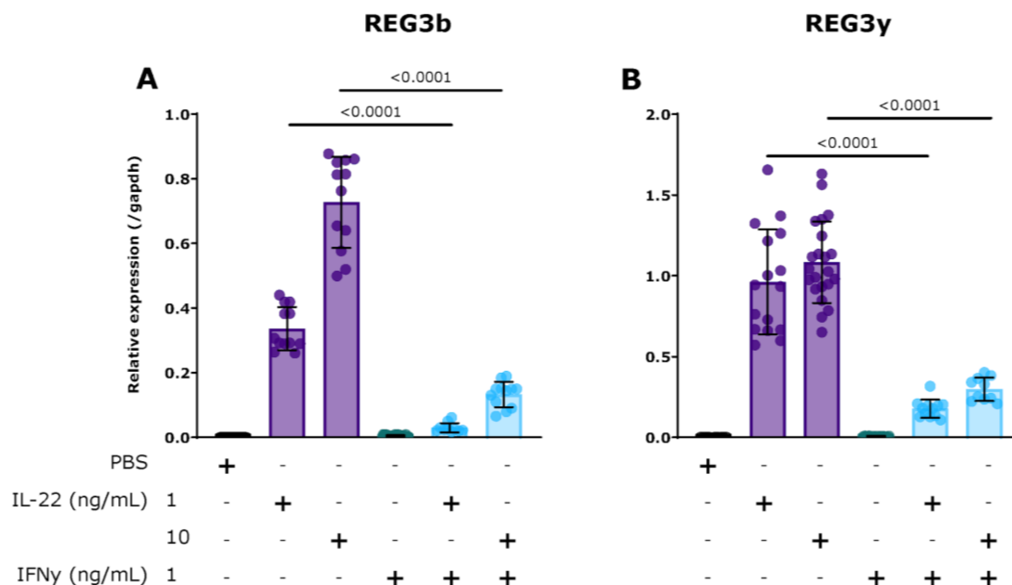

Figure 4.1. IL-22 and IFNy Co-stimulatory Effects on AMP Expression.
Organoids were stimulated for 24 hour and evaluated for gene expression by QPCR. (A,B) IFNy dampens IL-22 stimulated upregulation of AMPs. QPCR samples ran in technical duplicates and collected from >3 experiments. Ordinary one-way ANOVA followed by Tukey's multiple comparisons test and expressed as mean +/- SD.

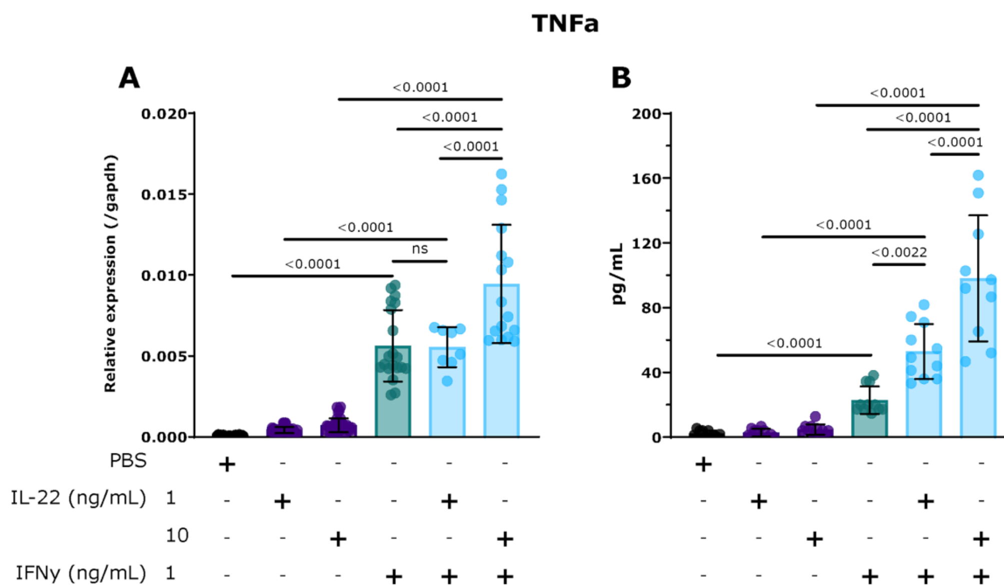

Figure 4.2. IL-22 and IFNy Co-stimulatory Effects on TNFa Gene Expression and Protein Induction.
Organoids were stimulated for 24 hours and evaluated for gene expression by QPCR. Protein expression was assessed at 48 hours via ELISA. (A) IL-22 amplifies IFNy-induced TNFa gene expression. (B) IL-22 amplifies IFNy induced TNFa protein levels, in supernatant, in a dose-dependent manner. QPCR samples were ran in technical duplicates and collected from >3 experiments. ELISA data point represent independent biological samples collected from ≥ 3 experiments. Ordinary one-way ANOVA followed by Tukey's multiple comparisons test and expressed as mean +/- SD.

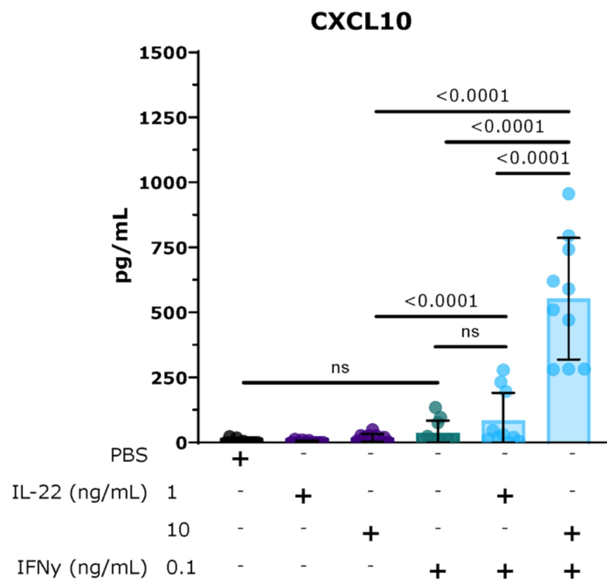

Figure 4.3. IFNy and IL-22 Co-stimulation on CXCL10 Protein Levels in Organoid Culture Media.
IL-22 and IFNy synergize increasing CXCL10 protein concentration in a dose-dependent manner. Data point represent independent biological samples collected from 4 experiments. Ordinary one-way ANOVA followed by Tukey’s multiple comparisons test and expressed as mean +/- SD.

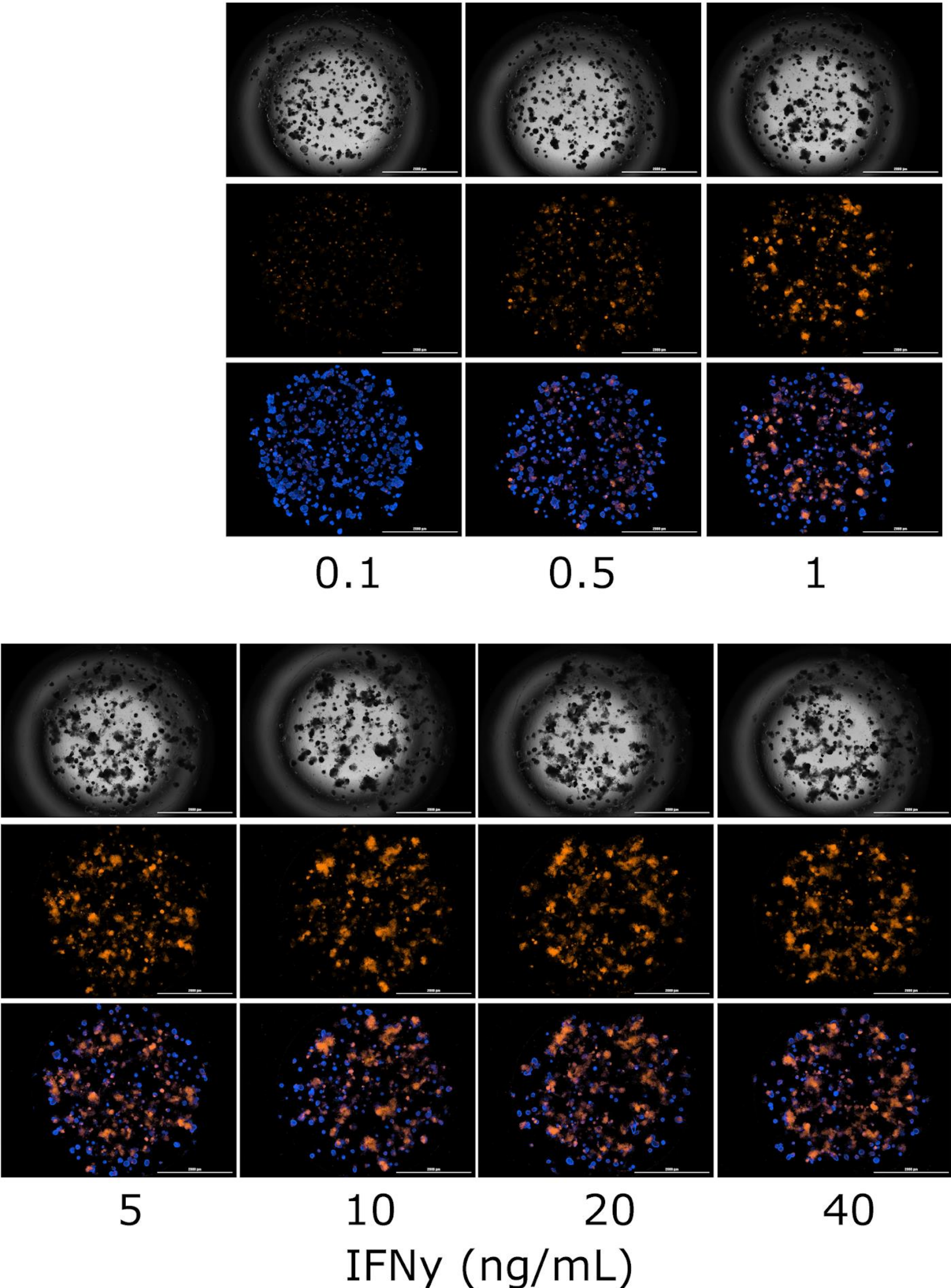

Figure 4.4. PI/H Image of Organoids Treated with IFNy at Different Concentrations for 48 Hours.

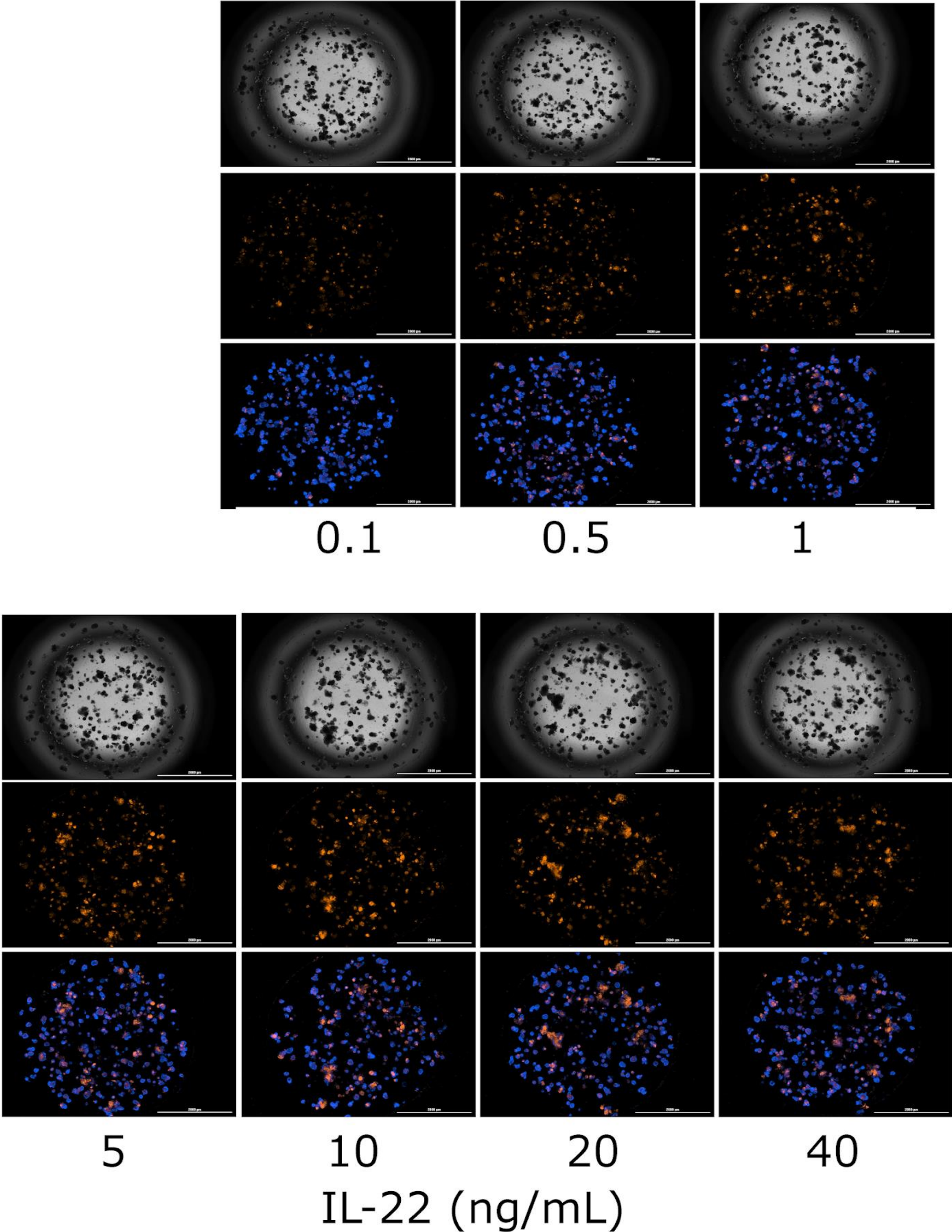

Figure 4.5. PI/H Images of Organoids Treated with IL-22 at Different Concentrations for 48 Hours.

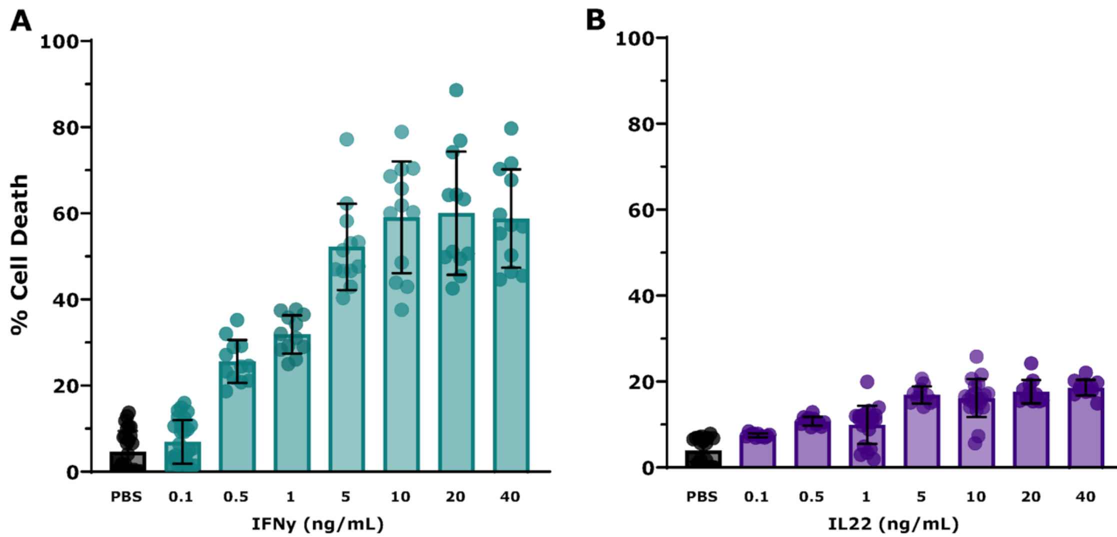

Figure 4.6. Cytokine Stimulation on Organoid Cell Viability.
SI organoids were stimulated for 48 hours then evaluated by PI/H for cell death (A,B) Increasing concentration of cytokine increases cell death. Data points are representative of one well and were collected over >3 experiments. Significance was determined using Ordinary one-way ANOVA followed by Tukey’s multiple comparisons test and expressed as mean +/- SD.

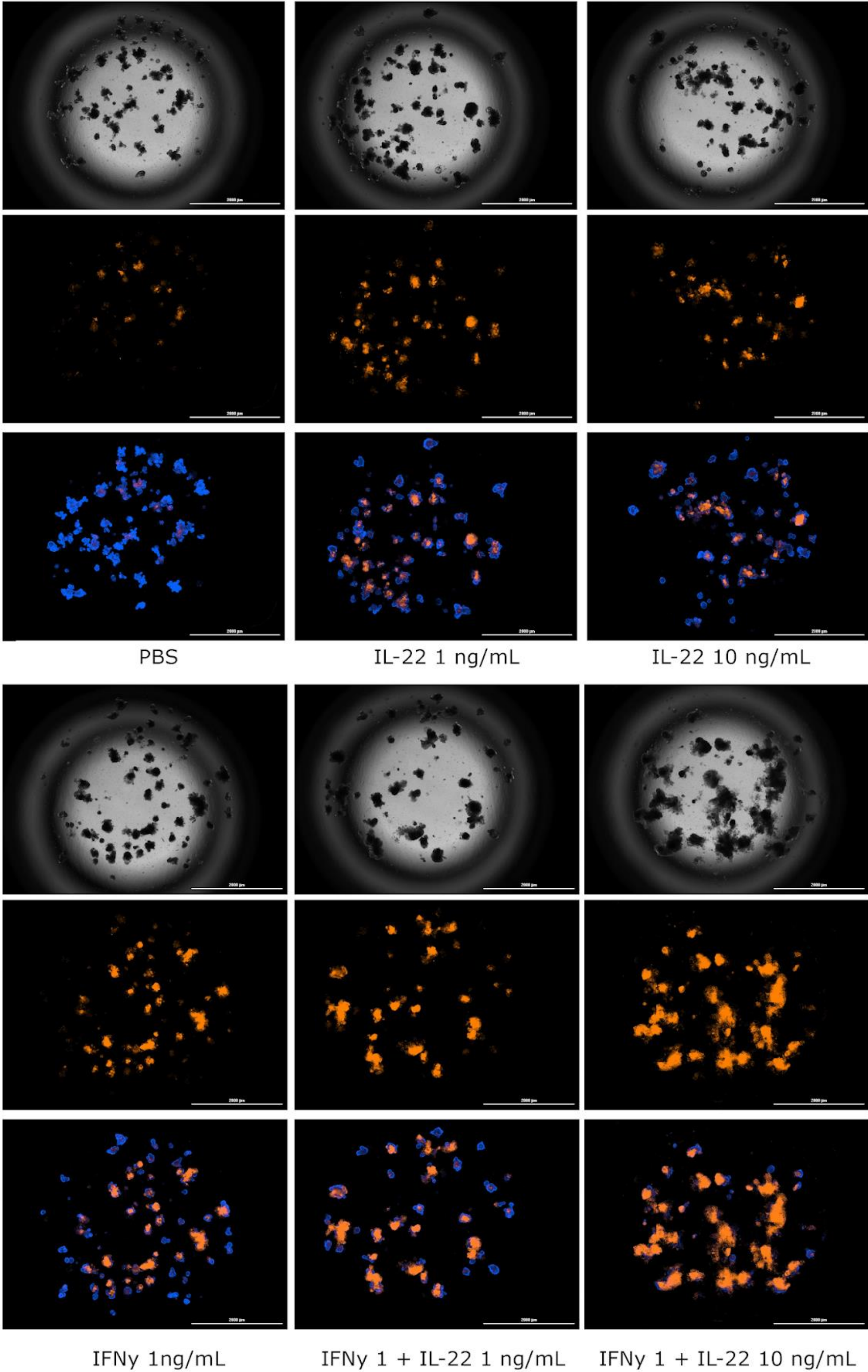

Figure 4.7. PI/H Images of Organoids After 48 Hours of Stimulation.

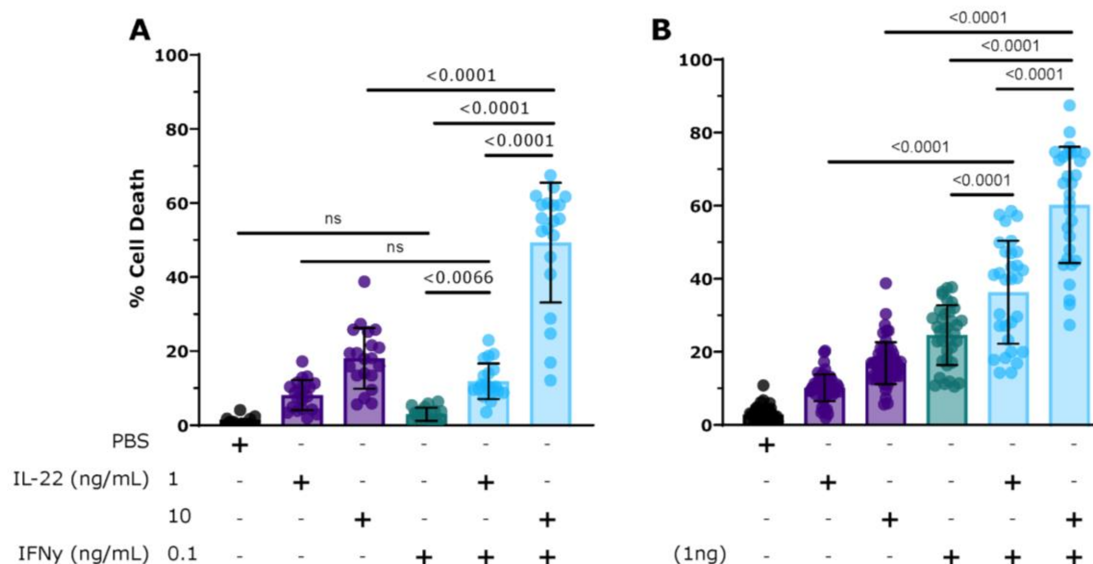

Figure 4.8. IFNy and IL-22 Co-stimulation on Cell Viability of Organoids.
SI organoids were stimulated for 48 hours then evaluated by PI/H for cell death. (A,B) IL-22 and IFNy synergize, enhancing cell death in organoids. Data points are representative of one well and were collected over ≥3 experiments. Significance was determined using Ordinary one-way ANOVA followed by Tukey's multiple comparisons test and expressed as mean +/- SD.

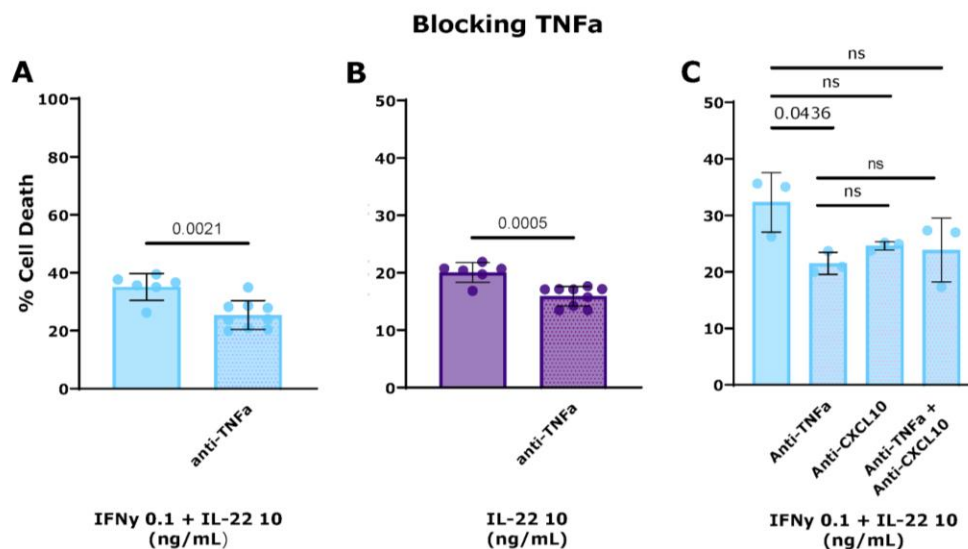

Figure 4.9. Anti-TNFa on Organoid Cell Viability.
SI organoids were stimulated with cytokines and anti-bodies for 48 hours then evaluated by PI/H for cell death. (A) anti-TNFa reduces cell death in organoids co-stimulated with IL-22 and IFNy. (B) anti-TNFa reduces cell death in organoids stimulated with 10ng/mL IL-22. (C) anti-CXCL10 does not significantly reduce cell death compared to untreated groups. Data points are representative of one well and were collected over ≥ 3 experiments (C's data is from 1 experiment). Significance was determined using Ordinary one-way ANOVA followed by Tukey's multiple comparisons test and expressed as mean +/- SD. Groups of two evaluated by T-tests.

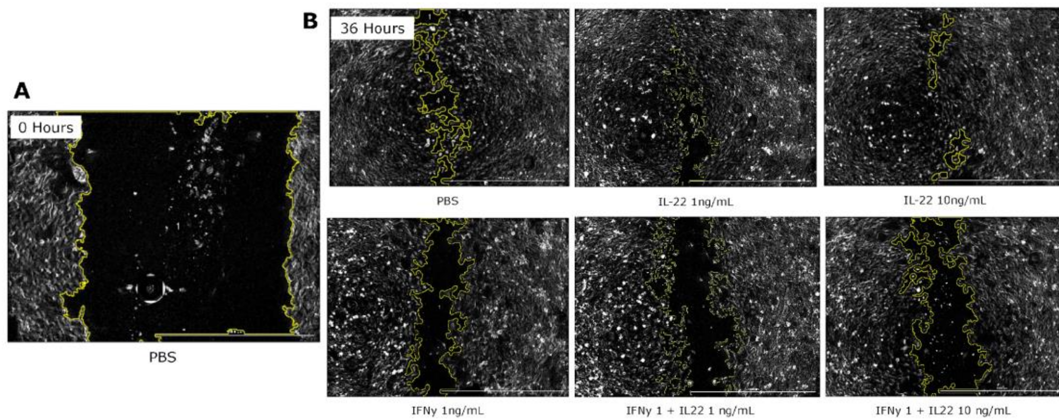

Figure 4.10. Digital Phase Contrast Images of Scratch Assay on Mode-k Cells.

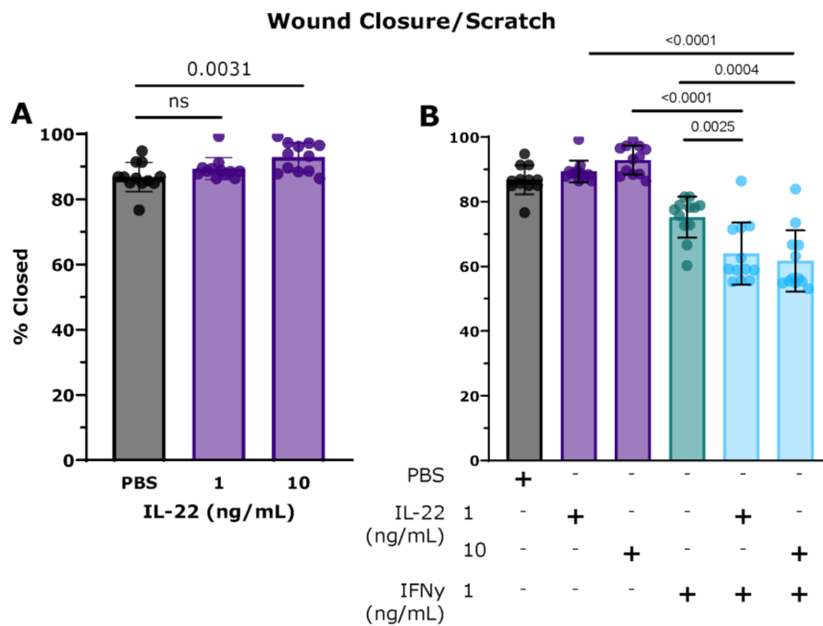

Figure 4.11. IL-22 and IFNy Co-stimulation in Wound Healing.
Scratch assay was performed on mode-k cells with a 24 hours of stimulation prior to (and during) the scratch and evaluation 36 hours post scratch. (A). Higher dose IL-22 accelerates wound. (B) IL-22 and IFNy co-stimulation slows wound closure compared to PBS. data points represent 1 well collected from 3 experiments. Significance was determined using Ordinary one-way ANOVA followed by Tukey's multiple comparisons test and expressed as mean +/- SD.

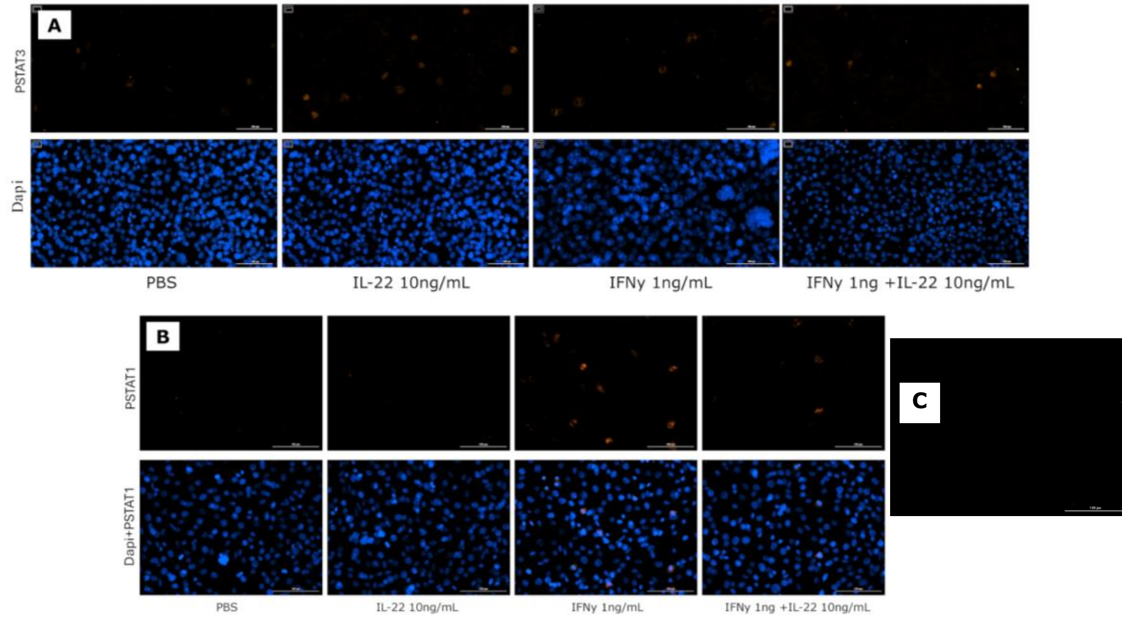

Figure 4.12. Immunofluorescence of Phospho-STATS in Mode-K Cells.
(A) PSTAT3 IF images (B) PSTAT1 IF images (C) Isotype control.

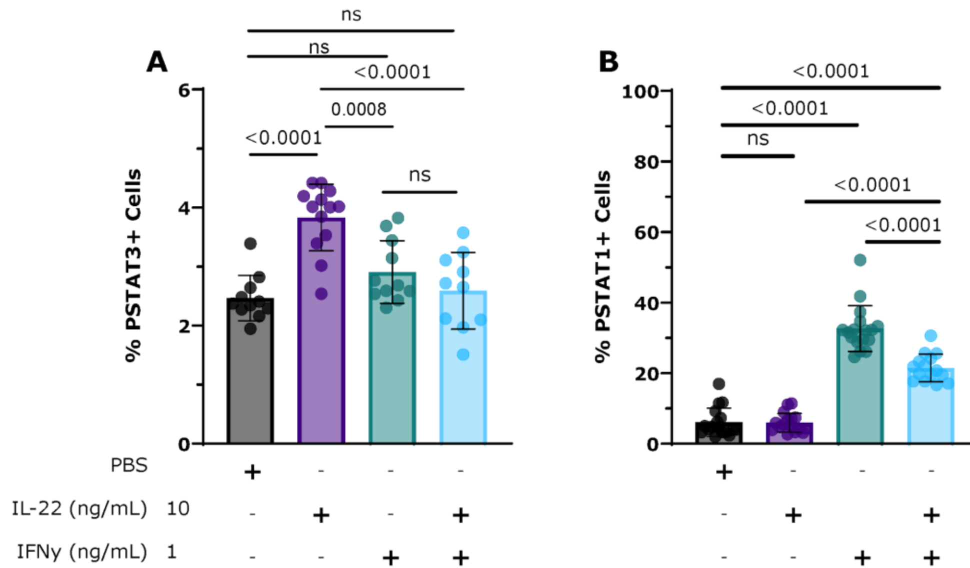

Figure 4.13. Mode-k Cells PSTATs in Response to Cytokine Stimulation.
Mode-k cells' PSTAT3 and PSTAT1 was evaluated by immunofluorescence after 24 hours of stimulation. stimulation (E). IL-22 increased PSTAT3. Co-stimulation of IL-22 with IFNy significantly reduced PSTAT3 levels, compared to Il-22 alone. (F) IFNy stimulation significantly increases PSTAT1. Co-stimulation with IL-22 significantly decreases PSTAT1, compared to IFNy alone. Data points representative of 1 image collected over 1 experiment for PSTAT3 and 2 for experiments PSTAT1. Significance was determined using Ordinary one-way ANOVA followed by Tukey's multiple comparisons test and expressed as mean +/- SD.

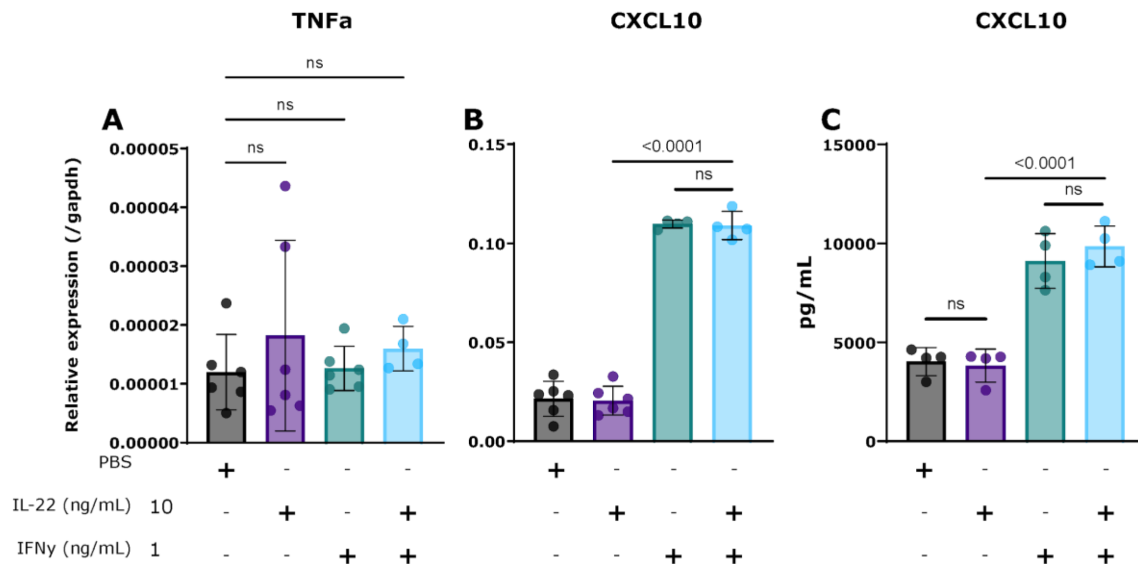

Figure 4.14. IL-22 and IFNy Stimulatione Effects on Mode-k Cells.
Mode-k cells were stimulated with cytokines for 24 hours and gene expression was evaluated by QPCR. (A) Mode-k cells do not express significant amounts of TNFa under any conditions. (B) IL-22 and IFNy and IL-22 co-stimulation does not synergistically increase CXCL10 gene expression. Mode-k cells constitutively express CXCL10. (C) IL-22 and IFNy and IL-22 co-stimulation does not synergistically increase CXCL10 protein concentration. Mode-k cells constitutively express CXCL10 at high levels. QPCR samples ran in technical duplicates and collected from 1 experiment. ELISA data points represent independent biological samples collected from 4 experiments. Ordinary one-way ANOVA followed by Tukey's multiple comparisons test and expressed as mean +/- SD.

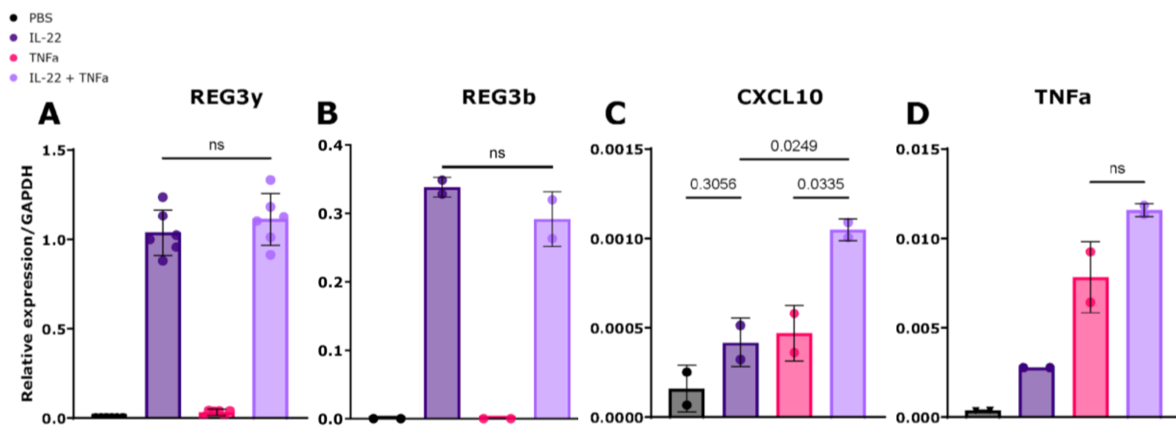

Figure 4.15. IL-22 and TNFa Co-stimulation of Organoids.
Organoids were stimulated for 24 hours and gene expression was evaluated by QPCR. (A,B) TNFa does not downregulate IL-22 stimulated AMP expression. (C.) TNFa and IL-22 co-stimulation synergistically upregulates CXCL10 expression. (D) TNFa and IL-22 co-stimulation did not enhance TNFa gene expression compared to TNFa alone. QPCR samples ran in technical duplicates and collected from 1 experiment. Ordinary one-way ANOVA followed by Tukey's multiple comparisons test and expressed as mean +/- SD.

3. Conclusion

This study investigated the interactions between IL-22 and IFNγ on epithelial cells, particularly concerning cytokine-driven gene expression and cellular responses, including cytotoxicity and wound healing. The findings reveal intricate interactions between these cytokines that affect cellular processes relevant to IBD pathogenesis.

IL-22 and IFNγ traditionally have opposing roles, with IL-22 typically promoting regenerative responses and IFNγ mediating inflammatory actions. This study shows that co-stimulation with IFNγ can convert the usually protective effects of IL-22 into a pro-inflammatory response.

IFNγ dampens IL-22-induced REG3b and REG3y expression, which is crucial for mucosal healing and antimicrobial defense. This interaction suggests a dominant inhibitory role of IFNγ over the beneficial effects of IL-22 in epithelial repair and maintenance. The synergistic effect of IL-22 and IFNγ on the induction of TNFα and CXCL10 further demonstrates the complexity of cytokine interactions. Surprisingly, higher doses of IL-22 further enhanced the induction of TNFa and CXCL10. The elevated levels of TNFa are notably problematic as elevated TNFa is one of the most prominent characteristics of IBD. TNFα synergizes with IFNγ, acting on epithelial cells which produce elevated levels of CXCL9, CXCL10, CXCL11, and TNFα. CXCL9, CXCL10, and CXCL11, through chemotaxis, attract CD8 cytotoxic T cells and NK cells, which produce IFNγ and TNFα, creating a feedforward loop that further promotes a pro-inflammatory environment (Suzuki, 2007; Dwinell;2001 Kulkarni 2017). CXCR3 and its ligand, particularly CXCL10, are being investigated as therapeutic targets in autoimmunity (Christen, 2003). Co-stimulation of IL-22 and IFNγ in mode-k cells did not

upregulate CXCL10 significantly from IFNγ alone. Mode-k cells also did not increase TNFa gene expression in response to any stimuli. IL-22 and TNFa co-stimulation of organoids did significantly upregulate CXCL10. These results suggest TNFa is an integral component of upregulation of CXCL10 in the co-stimulatory conditions.

The cytotoxic effects induced by co-stimulation with IFNy and IL-22 in intestinal organoids and the partial mitigation of these effects by anti-TNFα treatment underscore TNFα's critical role in cytokine-induced epithelial damage. This finding is consistent with the known pro-apoptotic functions of TNFα and supports ongoing therapeutic strategies targeting TNFα in IBD. The lack of significant impact of anti-CXCL10 treatment on cell viability indicates a secondary role of CXCL10 in cytokine-induced cytotoxicity despite its upregulation in inflammatory conditions.

In the context of epithelial barrier integrity, the inhibition of wound closure by IFNγ highlights the challenges in managing IBD, where healing of mucosal lesions is crucial. IFNy and IL-22 co-stimulation further inhibiting wound closure is particularly problematic, as IL-22 is critical in wound healing.

Lastly, the alterations in STAT signaling induced by cytokine co-stimulation provide a molecular basis for the observed cellular responses. The reduction of PSTAT3-positive cells in the presence of IFNγ, even with high levels of IL-22, reveals a competitive antagonism between these cytokines at the signaling level. Conversely, the decrease in PSTAT1 expression with IL-22 addition suggests a partial counter-regulatory mechanism, though insufficient to reverse IFNγ effects fully. The reduction of STAT1 without a reduction in CXCL10 gene transcription or protein induction could indicate CXCL10 gene transcription is primarily using another pathway, such as PI3K.

An essential insight from this study, with significant therapeutic implications, is the critical role of cytokine dosage. We observed that problematic interactions between these cytokines predominantly occurred at higher concentrations and in combination. This underscores the importance of not only discerning the interactions between cytokines but also understanding how these interactions vary with concentration. Such knowledge can refine our approaches to treatment. This nuanced understanding of dosage effects highlights the need for precision in cytokine modulation strategies in clinical and research settings. These findings also demonstrate that IL-22, typically seen as an anti-inflammatory agent, can assume a pro-inflammatory role when combined with IFNγ, complicating its effects on epithelial cells. This study highlights the need to consider specific cytokine interactions in developing more effective IBD treatments.

4. References

1. Abdel-Rahman, L. I. H., & Morgan, X. C. (2023). Searching for a Consensus Among Inflammatory Bowel Disease Studies: A Systematic Meta-Analysis. *Inflammatory Bowel Diseases*, 29(1), 125-139. https://doi.org/10.1093/ibd/izac194

2. Abraham, C., & Cho, J. H. (2009). Inflammatory bowel disease. *The New England Journal of Medicine, 361*(21), 2066-2078. https://doi.org/10.1056/NEJMra0804647

3. Aparicio-Domingo, P., Romera-Hernández, M., Karrich, J. J., Cornelissen, F., Papazian, N., Lindenbergh-Kortleve, D. J., Butler, J. A., Boon, L., Coles, M. C., Samsom, J. N., & Cupedo, T. (2015). Type 3 innate lymphoid cells maintain intestinal epithelial stem cells after tissue damage. *Journal of Experimental Medicine, 212*(11), 1783-1791. https://doi.org/10.1084/jem.20150318

4. Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., et al. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*.

5. Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., et al. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*, 500, 232–236.

6. Bergemalm, D., Andersson, E., Hultdin, J., Eriksson, C., Rush, S. T., Kalla, R., Adams, A. T., Keita, Å. V., D'Amato, M., Gomollon, F., Jahnsen, J., (IBD Character Consortium), Ricanek, P., Satsangi, J., Repsilber, D., Karling, P., & Halfvarson, J. (2021). Systemic Inflammation in Preclinical Ulcerative Colitis.

Gastroenterology, 161(5), 1526–1539.e9.
https://doi.org/10.1053/j.gastro.2021.07.026

7. Billmeier, U., Dieterich, W., Neurath, M. F., & Atreya, R. (2016). Molecular mechanism of action of anti-tumor necrosis factor antibodies in inflammatory bowel diseases. *World Journal of Gastroenterology*, 22(42), 9300–9313. https://doi.org/10.3748/wjg.v22.i42.9300

8.Bode KJ, Mueller S, Schweinlin M, Metzger M, Brunner T. A fast and simple fluorometric method to detect cell death in 3D intestinal organoids. Biotechniques. 2019 Jul;67(1):23-28. doi: 10.2144/btn-2019-0023. Epub 2019 Jun 20. PMID: 31218886.

9. Breugelmans, T., Arras, W., Oosterlinck, B., Jauregui-Amezaga, A., Somers, M., Cuypers, B., Laukens, K., De Man, J. G., De Schepper, H. U., De Winter, B. Y., Smet, A. (2023). IL-22-activated MUC13 impacts on colonic barrier function through JAK1/STAT3, SNAI1/ZEB1 and ROCK2/MAPK signaling. *Cells, 12*(9), 1224. https://doi.org/10.3390/cells12091224

10. Buckley, A., & Turner, J. R. (2018). Cell biology of tight junction barrier regulation and mucosal disease. *Cold Spring Harbor Perspectives in Biology*, 10(1), a029314. https://doi.org/10.1101/cshperspect.a029314

11. Buhner, S., Buning, C., Genschel, J., et al. (2006). Genetic basis for increased intestinal permeability in families with Crohn's disease: role of CARD15 3020insC mutation? *Gut, 55*, 342–347. https://doi.org/10.1136/gut.2005.065300

12. Buonocore, S., Ahern, P. P., Uhlig, H. H., Ivanov, I. I., Littman, D. R., Maloy, K. J., & Powrie, F. (2010). Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature*, 464(7293), 1371–1375. https://doi.org/10.1038/nature08949

13. Carreras-Torres, R., Ibáñez-Sanz, G., Obón-Santacana, M., et al. (2020). Identifying environmental risk factors for inflammatory bowel diseases: a Mendelian randomization study. *Scientific Reports*, 10, 19273. https://doi.org/10.1038/s41598-020-76361-2

14. Chen, G. Y., Shaw, M. H., Redondo, G., & Núñez, G. (2008). The innate immune receptor Nod1 protects the intestine from inflammation-induced tumorigenesis. *Cancer Research*, 68, 10060–10067.

15. Chiba M, Nakane K, Komatsu M. (2019). Westernized Diet is the Most Ubiquitous Environmental Factor in Inflammatory Bowel Disease. *Perm J*, 23:18-107. doi: 10.7812/TPP/18-107. PMID: 30624192; PMCID: PMC6326567.

16. Christen, U., McGavern, D. B., Luster, A. D., von Herrath, M. G., & Oldstone, M. B. (2003). Among CXCR3 chemokines, IFN-gamma-inducible protein of 10 kDa (CXC chemokine ligand (CXCL) 10) but not monokine induced by IFN-gamma (CXCL9) imprints a pattern for the subsequent development of autoimmune disease. *Journal of Immunology*, 171, 6838–6845.

17.Clark E, Hoare C, Tanianis-Hughes J, Carlson GL, Warhurst G. Interferon gamma induces translocation of commensal Escherichia coli across gut epithelial cells via a lipid raft-mediated process. Gastroenterology. 2005 May;128(5):1258-67. doi: 10.1053/j.gastro.2005.01.046. PMID: 15887109.

18. Cohen, L. J., Cho, J. H., Gevers, D., & Chu, H. (2019). Genetic Factors and the Intestinal Microbiome Guide Development of Microbe-Based Therapies for Inflammatory Bowel Diseases. *Gastroenterology*, 156, 2174-2189. https://doi.org/10.1053/j.gastro.2019.03.017

19. Colombel, J. F., Sandborn, W. J., Reinisch, W., Mantzaris, G. J., Kornbluth, A., Rachmilewitz, D., Lichtiger, S., D'Haens, G., Diamond, R. H., Broussard, D. L., et al. (2010). Infliximab, azathioprine, or combination therapy for Crohn's disease. *New England Journal of Medicine*, 362, 1383–1395. https://doi.org/10.1056/NEJMoa0904492

20. Conlon, T. M., Knolle, P. A., & Yildirim, A. Ö. (2021). Local tissue development of type 1 innate lymphoid cells: guided by interferon-gamma. *Signal Transduction and Targeted Therapy*, 6, 287. https://doi.org/10.1038/s41392-021-00705-1

21. Consortium UIG, Barrett, J. C., Lee, J. C., et al. (2009). Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nature Genetics*, 41, 1330–1334. https://doi.org/10.1038/ng.482

22. Coombes, J. L., Siddiqui, K. R., Arancibia-Cárcamo, C. V., Hall, J., Sun, C. M., Belkaid, Y., & Powrie, F. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *The Journal of Experimental Medicine, 204*(8), 1757-1764. https://doi.org/10.1084/jem.20070590

23. Dwinell, M. B., Lügering, N., Eckmann, L., & Kagnoff, M. F. (2001). Regulated production of interferon-inducible T-cell chemoattractants by human intestinal epithelial cells. *Gastroenterology*, 120, 49–59.

24. Ey, B., Eyking, A., Gerken, G., Podolsky, D. K., & Cario, E. (2009). TLR2 mediates gap junctional intercellular communication through connexin-43 in intestinal epithelial barrier injury. *Journal of Biological Chemistry*, 284, 22332–22343.

25. Fish SM, Proujansky R, Reenstra WW. Synergistic effects of interferon gamma and tumour necrosis factor alpha on T84 cell function. Gut. 1999 Aug;45(2):191-8. doi: 10.1136/gut.45.2.191. PMID: 10403730; PMCID: PMC1727614.

26. Foerster, E. G., Mukherjee, T., Cabral-Fernandes, L., Rocha, J. D. B., Girardin, S. E., & Philpott, D. J. (2022). How autophagy controls the intestinal epithelial barrier. *Autophagy*, 18(1), 86-103. https://doi.org/10.1080/15548627.2021.1909406. Epub 2021 Apr 27. PMID: 33906557; PMCID: PMC8865220.

27. Gao, S., Li, Y., Wu, D., Jiao, N., Yang, L., Zhao, R., Xu, Z., Chen, W., Lin, X., Cheng, S., Zhu, L., Lan, P., & Zhu, R. (2022). IBD Subtype-Regulators IFNG and GBP5 Identified by Causal Inference Drive More Intense Innate Immunity and Inflammatory Responses in CD Than Those in UC. *Frontiers in Pharmacology*, 13, 869200. https://doi.org/10.3389/fphar.2022.869200

28. Ghosh, S., Whitley, C. S., Haribabu, B., & Jala, V. R. (2021). Regulation of Intestinal Barrier Function by Microbial Metabolites. *Cellular and Molecular Gastroenterology and Hepatology*, 11(5), 1463-1482. https://doi.org/10.1016/j.jcmgh.2021.02.007

29. Gieryńska, M., Szulc-Dąbrowska, L., Struzik, J., Mielcarska, M. B., & Gregorczyk-Zboroch, K. P. (2022). Integrity of the intestinal barrier: The involvement of

epithelial cells and microbiota—A mutual relationship. *Animals*, 12(2), 145. https://doi.org/10.3390/ani12020145

30. Gonsky, R., Deem, R. L., Landers, C. J., Haritunians, T., Yang, S., & Targan, S. R. (2014). IFNG rs1861494 polymorphism is associated with IBD disease severity and functional changes in both IFNG methylation and protein secretion. *Inflammatory Bowel Diseases*, 20(10), 1794-801. https://doi.org/10.1097/MIB.0000000000000172

31. Goto, Y., Uematsu, S., & Kiyono, H. (2016). Epithelial glycosylation in gut homeostasis and inflammation. *Nature Immunology*, 17, 1244–1251.

32. Gunasekera, D. C., Ma, J., Vacharathit, V., Shah, P., Ramakrishnan, A., Uprety, P., Shen, Z., Sheh, A., Brayton, C. F., Whary, M. T., Fox, J. G., Bream, J. H. (2020). the development of colitis in Il10-/- mice is dependent on IL-22. *Mucosal Immunology, 13*(3), 493-506. https://doi.org/10.1038/s41385-019-0252-3

33. Günther, C., Martini, E., Wittkopf, N., Amann, K., Weigmann, B., Neumann, H., Waldner, M. J., Hedrick, S. M., Tenzer, S., Neurath, M. F., et al. (2011 Caspase-8 regulates TNF-alpha-induced epithelial necroptosis and terminal ileitis. *Nature*, 477, 335–339. https://doi.org/10.1038/nature10400

34. Han, X., Lee, A., Huang, S., Gao, J., Spence, J. R., Owyang, C. (2019). Lactobacillus rhamnosus GG prevents epithelial barrier dysfunction induced by interferon-gamma and fecal supernatants from irritable bowel syndrome patients in human intestinal enteroids and colonoids. *Gut Microbes, 10*(1), 59-76. https://doi.org/10.1080/19490976.2018.1479625

35. Hu Y, Chen Z, Xu C, Kan S, Chen D. Disturbances of the Gut Microbiota and Microbiota-Derived Metabolites in Inflammatory Bowel Disease. Nutrients. 2022 Dec 2;14(23):5140. doi: 10.3390/nu14235140. PMID: 36501169; PMCID: PMC9735443

36. Ito, R., Shin-Ya, M., Kishida, T., Urano, A., Takada, R., Sakagami, J., Imanishi, J., Kita, M., Ueda, Y., Iwakura, Y., Kataoka, K., Okanoue, T., Mazda, O. (2006). Interferon-gamma is causatively involved in experimental inflammatory bowel disease in mice. *Clinical and Experimental Immunology, 146*(2), 330-338. https://doi.org/10.1111/j.1365-2249.2006.03214.x

37. Jarmakiewicz-Czaja, S., Zielińska, M., Sokal, A., & Filip, R. (2022). Genetic and Epigenetic Etiology of Inflammatory Bowel Disease: An Update. *Genes*, 13(12), 2388. https://doi.org/10.3390/genes13122388

38. Kedia, S., Virmani, S., K Vuyyuru, S., et al. (2022). Faecal microbiota transplantation with anti-inflammatory diet (FMT-AID) followed by anti-inflammatory diet alone is effective in inducing and maintaining remission over 1 year in mild to moderate ulcerative colitis: a randomised controlled trial. *Gut*, 71, 2401-2413.

39. Keir, M., Yi, Y., Lu, T., & Ghilardi, N. (2020). The role of IL-22 in intestinal health and disease. *Journal of Experimental Medicine, 217*(3), e20192195. https://doi.org/10.1084/jem.20192195

40. Kim, S., Nowakowska, A., Kim, Y. B., Shin, H. Y. (2022). Integrated CRISPR-Cas9 System-Mediated Knockout of IFN-γ and IFN-γ Receptor 1 in the Vero Cell Line

Promotes Viral Susceptibility. *International Journal of Molecular Sciences*, 23(15), 8217. https://doi.org/10.3390/ijms23158217

41. Kulkarni, N., Manisha, P., & Girdhari, L. (2017). Role of chemokine receptors and intestinal epithelial cells in the mucosal inflammation and tolerance. *Journal of Leukocyte Biology*, 101, 377–394.
42. Kumar, M., Murugesan, S., Ibrahim, N., et al. (2024). Predictive biomarkers for anti-TNF alpha therapy in IBD patients. *Journal of Translational Medicine, 22*, 284. https://doi.org/10.1186/s12967-024-05058-1
43. Kwon, Y. H., Wang, H., Denou, E., Ghia, J. E., Rossi, L., Fontes, M. E., et al. (2019). Modulation of gut microbiota composition by serotonin signaling influences intestinal immune response and susceptibility to colitis. *Cellular and Molecular Gastroenterology and Hepatology*, 7, 709–728.
44. Kyo, K., Parkes, M., Takei, Y., et al. (1999). Association of ulcerative colitis with rare VNTR alleles of the human intestinal mucin gene, MUC3. *Human Molecular Genetics*, 8, 307–311. https://doi.org/10.1093/hmg/8.2.307
45. Larkin, J. 3rd, Ahmed, C. M., Wilson, T. D., & Johnson, H. M. (2013). Regulation of interferon gamma signaling by suppressors of cytokine signaling and regulatory T cells. *Frontiers in Immunology*, 4, 469. https://doi.org/10.3389/fimmu.2013.00469
46. Lavoie, S., Conway, K. L., Lassen, K. G., Jijon, H. B., Pan, H., Chun, E., Michaud, M., Lang, J. K., Comeau, C. A. G., Dreyfuss, J., et al. (2019). The Crohn's disease polymorphism, ATG16L1 T300A, alters the gut microbiota and enhances

the local Th1/Th17 response. *eLife*, 8, e39982. https://doi.org/10.7554/eLife.39982

47. Linares, R., Francés, R., Gutiérrez, A., & Juanola, O. (2021). Bacterial Translocation as Inflammatory Driver in Crohn's Disease. *Frontiers in Cell and Developmental Biology*, 9, 703310. https://doi.org/10.3389/fcell.2021.703310
48. Liu, S., da Cunha, A. P., Rezende, R. M., Cialic, R., Wei, Z., Bry, L., et al. (2016). The host shapes the gut microbiota via fecal MicroRNA. *Cell Host & Microbe*, 19, 32–
49. Marchiando, A. M., et al. (2010). Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. *Journal of Cell Biology*, 189, 111–126. https://doi.org/10.1083/jcb.200902153
50. Martinez, J. E., Kahana, D. D., Ghuman, S., Wilson, H. P., Wilson, J., Kim, S. C. J., Lagishetty, V., Jacobs, J. P., Sinha-Hikim, A. P., & Friedman, T. C. (2021). Unhealthy lifestyle and gut dysbiosis: A better understanding of the effects of poor diet and nicotine on the intestinal microbiome. *Frontiers in Endocrinology, 12*, Article 667066. https://doi.org/10.3389/fendo.2021.667066
51. Martinez-Medina, M., Denizot, J., Dreux, N., et al. (2014). Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function flavouring AIEC colonisation. *Gut, 63*, 116-124. https://doi.org/10.1136/gutjnl-2013-304909
52. McDowell C, Farooq U, Haseeb M. Inflammatory Bowel Disease. [Updated 2023 Aug 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470312/

53. McCole, D. F. (2014). IBD candidate genes and intestinal barrier regulation. *Inflammatory Bowel Diseases*, 20(10), 1829-1849. https://doi.org/10.1097/MIB.0000000000000090, PMID: 25215613; PMCID: PMC4357271.

54. Mizoguchi, A., Yano, A., Himuro, H., et al. (2018). Clinical importance of IL-22 cascade in IBD. *Journal of Gastroenterology, 53*, 465–474. https://doi.org/10.1007/s00535-017-1401-7

55. Muise, A. M., Walters, T. D., Glowacka, W. K., et al. (2009). Polymorphisms in E-cadherin (CDH1) result in a mis-localised cytoplasmic protein that is associated with Crohn’s disease. *Gut*, 58, 1121–1127. https://doi.org/10.1136/gut.2008.169136

56. Murray, P. D., McGavern, D. B., Pease, L. R., & Rodriguez, M. (2002). Cellular sources and targets of IFN-gamma-mediated protection against viral demyelination and neurological deficits. *European Journal of Immunology*, 32(3), 606-615. https://doi.org/10.1002/1521-4141(200203)32:3<606::AID-IMMU606>3.0.CO;2-D

57. National Institutes of Health (NIH). (n.d.). Overweight & Obesity Statistics. Retrieved from https://www.niddk.nih.gov/health-information/health-statistics/overweight-obesity

58. Nava, P., Koch, S., Laukoetter, M. G., Lee, W. Y., Kolegraff, K., Capaldo, C. T., Beeman, N., Addis, C., Gerner-Smidt, K., Neumaier, I., Skerra, A., Li, L., Parkos, C. A., & Nusrat, A. (2010). Interferon-gamma regulates intestinal epithelial

homeostasis through converging beta-catenin signaling pathways. *Immunity*, 32(3), 392–402. https://doi.org/10.1016/j.immuni.2010.03.001

59. Nenci, A., Becker, C., Wullaert, A., Gareus, R., van Loo, G., Danese, S., Huth, M., Nikolaev, A., Neufert, C., Madison, B., et al. (2007). Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature*, 446, 557–561. https://doi.org/10.1038/nature05698
60. Neufert, C., Pickert, G., Zheng, Y., Wittkopf, N., Warntjen, M., Nikolaev, A., Ouyang, W., Neurath, M. F., & Becker, C. (2010). Activation of epithelial STAT3 regulates intestinal homeostasis. *Cell Cycle, 9*(4), 652-655. https://doi.org/10.4161/cc.9.4.10615
61. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JCY, Chan FKL, Sung JJY, Kaplan GG. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2017 Dec 23;390(10114):2769-2778. doi: 10.1016/S0140-6736(17)32448-0. Epub 2017 Oct 16. Erratum in: Lancet. 2020 Oct 3;396(10256):e56. PMID: 29050646.
62. Odenwald, M. A., & Turner, J. R. (2017). The intestinal epithelial barrier: a therapeutic target? *Nature Reviews Gastroenterology & Hepatology*, 14(1), 9–21. https://doi.org/10.1038/nrgastro.2016.169
63. Otani, T., & Furuse, M. (2020). Tight junction structure and function revisited. *Trends in Cell Biology*, 30(10), 805–817. https://doi.org/10.1016/j.tcb.2020.08.004
64. OpenAI. (2024). ChatGPT [Large language model]. https://chat.openai.com/chat.

65. Palmela, C., Chevarin, C., Xu, Z., et al. (2018). Adherent-invasive Escherichia coli in inflammatory bowel disease. *Gut, 67*, 574-587. https://doi.org/10.1136/gutjnl-2017-314903

66. Panaccione, R. (2013). Mechanisms of inflammatory bowel disease. *Gastroenterology & Hepatology*, 9(8), 529-532. PMID: 24719603; PMCID: PMC3980998.

67. Pavlidis, P., Tsakmaki, A., Treveil, A., Li, K., Cozzetto, D., Yang, F., Niazi, U., Hayee, B. H., Saqi, M., Friedman, J., Korcsmaros, T., Bewick, G., Powell, N. (2022). Cytokine responsive networks in human colonic epithelial organoids unveil a molecular classification of inflammatory bowel disease. *Cell Reports*, 40(13), 111439. https://doi.org/10.1016/j.celrep.2022.111439

68. Perez-Toledo, M., Beristain-Covarrubias, N., Channell, W. M., Hitchcock, J. R., Cook, C. N., Coughlan, R. E., Bobat, S., Jones, N. D., Nakamura, K., Ross, E. A., Rossiter, A. E., Rooke, J., Garcia-Gimenez, A., Jossi, S., Persaud, R. R., Marcial-Juarez, E., Flores-Langarica, A., Henderson, I. R., Withers, D. R., Watson, S. P., & Cunningham, A. F. (2020). Mice Deficient in T-bet Form Inducible NO Synthase-Positive Granulomas That Fail to Constrain Salmonella. *The Journal of Immunology*, 205(3), 708–719. https://doi.org/10.4049/jimmunol.2000089

69. Pickert, G., Neufert, C., Leppkes, M., Zheng, Y., Wittkopf, N., Warntjen, M., Lehr, H. A., Hirth, S., Weigmann, B., Wirtz, S., Ouyang, W., Neurath, M. F., & Becker, C. (2009). STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound

healing. *Journal of Experimental Medicine, 206*(7), 1465-1472. https://doi.org/10.1084/jem.20082683

70. Platanias, L. (2005). Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nature Reviews Immunology*, 5, 375–386. https://doi.org/10.1038/nri1604

71. Price, A. E., Shamardani, K., Lugo, K. A., Deguine, J., Roberts, A. W., Lee, B. L., Barton, G. M. (2018). A map of Toll-like receptor expression in the intestinal epithelium reveals distinct spatial, cell type-specific, and temporal patterns. *Immunity, 49*(3), 560-575.e6. https://doi.org/10.1016/j.immuni.2018.07.016

72. Qiu, P., Ishimoto, T., Fu, L., Zhang, J., Zhang, Z., & Liu, Y. (2022). The gut microbiota in inflammatory bowel disease. *Frontiers in Cellular and Infection Microbiology, 12*, Article 733992. https://doi.org/10.3389/fcimb.2022.733992

73. Roda, G., Ng, S. C., Kotze, P. G., et al. (2020). Crohn’s disease. *Nature Reviews Disease Primers*, 6(22). https://doi.org/10.1038/s41572-020-0156-2

73.5. Ruder, B., Atreya, R., & Becker, C. (2019). Tumor necrosis factor alpha in intestinal homeostasis and gut related diseases. *International Journal of Molecular Sciences*, 20(8), 1887. https://doi.org/10.3390/ijms20081887

74. Santana, P. T., Rosas, S. L. B., Ribeiro, B. E., Marinho, Y., & de Souza, H. S. P. (2022). Dysbiosis in Inflammatory Bowel Disease: Pathogenic Role and Potential Therapeutic Targets. *International Journal of Molecular Sciences*, 23(7), 3464. https://doi.org/10.3390/ijms23073464

75. Salzman, N. H., Hung, K., Haribhai, D., Chu, H., Karlsson-Sjöberg, J., Amir, E., et al. (2010). Enteric defensins are essential regulators of intestinal microbial ecology. *Nature Immunology*, 11, 76–83.

76. Scharl, M., Paul, G., Weber, A., et al. (2009). Protection of epithelial barrier function by the Crohn's disease associated gene protein tyrosine phosphatase n2. *Gastroenterology*, 137, 2030–2040.e2035. https://doi.org/10.1053/j.gastro.2009.08.041

72. Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., et al. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*, 341, 569–573.

77. Singh L, Arora SK, Bakshi DK, Majumdar S, Wig JD. Potential role of CXCL10 in the induction of cell injury and mitochondrial dysfunction. Int J Exp Pathol. 2010 Jun;91(3):210-23. doi: 10.1111/j.1365-2613.2009.00697.x. Epub 2009 Dec 22. PMID: 20041963; PMCID: PMC2884089.

78. Soderholm, A. T., & Pedicord, V. A. (2019). Intestinal epithelial cells: at the interface of the microbiota and mucosal immunity. *Immunology*, 158(4), 267–280. https://doi.org/10.1111/imm.13117

79. Suenaert, P., et al. (2002). Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. *American Journal of Gastroenterology*, 97, 2000–2004. https://doi.org/10.1111/j.1572-0241.2002.05914.x

80. Suzuki, K., Kawauchi, Y., Palaniyandi, S. S., Veeraveedu, P. T., Fujii, M., Yamagiwa, S., Yoneyama, H., Han, G. D., Kawachi, H., Okada, Y., Ajioka, Y., Watanabe, K., Hosono, M., Asakura, H., Aoyagi, Y., & Narumi, S. (2007).

Blockade of interferon-gamma-inducible protein-10 attenuates chronic experimental colitis by blocking cellular trafficking and protecting intestinal epithelial cells. *Pathology International*, 57, 413–420.

81. Thiemann, S., Smit, N., Roy, U., Lesker, T. R., Gálvez, E. J. C., Helmecke, J., Basic, M., Bleich, A., Goodman, A. L., Kalinke, U., Flavell, R. A., Erhardt, M., & Strowig, T. (2017). Enhancement of IFNγ Production by Distinct Commensals Ameliorates Salmonella-Induced Disease. *Cell Host & Microbe*, 21(6), 682–694.e5. https://doi.org/10.1016/j.chom.2017.05.005
82. Trivedi, P. J., & Adams, D. H. (2018). Chemokines and Chemokine Receptors as Therapeutic Targets in Inflammatory Bowel Disease; Pitfalls and Promise. *Journal of Crohn's and Colitis*, 12(suppl_2), S641–S652. https://doi.org/10.1093/ecco-jcc/jjx145
83. Uguccioni, M., Gionchetti, P., Robbiani, D. F., et al. (1999). Increased expression of IP-10, IL-8, MCP-1, and MCP-3 in ulcerative colitis. *The American Journal of Pathology*, 155, 331–336.
84. Van den Steen, P., Rudd, P. M., Dwek, R. A., et al. (1998). Concepts and principles of O-linked glycosylation. *Critical Reviews in Biochemistry and Molecular Biology, 33*, 151–208. https://doi.org/10.1080/10409239891204198
85. Van Der Kraak, L. A., Schneider, C., Dang, V., Burr, A. H. P., Weiss, E. S., Varghese, J. A., Yang, L., Hand, T. W., Canna, S. W. (2021). Genetic and commensal induction of IL-18 drive intestinal epithelial MHCII via IFNγ. *Mucosal Immunology*, 14(5), 1100–1112. https://doi.org/10.1038/s41385-021-00419

86. Vich Vila, A., Imhann, F., Collij, V., Jankipersadsing, S. A., Gurry, T., Mujagic, Z., ... & others. (2018). Gut microbiota composition and functional changes in inflammatory bowel disease and irritable bowel syndrome. *Science Translational Medicine, 10*(472), eaap8914. https://doi.org/10.1126/scitranslmed.aap8914

87. Wei, H.-X., Wang, B., & Li, B. (2020). IL-10 and IL-22 in mucosal immunity: Driving protection and pathology. *Frontiers in Immunology, 11*, Article 1315. https://doi.org/10.3389/fimmu.2020.01315

88. Woznicki, J. A., Saini, N., Flood, P., Rajaram, S., Lee, C. M., Stamou, P., Skowyra, A., Bustamante-Garrido, M., Regazzoni, K., Crawford, N., McDade, S. S., Longley, D. B., Aza-Blanc, P., Shanahan, F., Zulquernain, S. A., McCarthy, J., Melgar, S., McRae, B. L., Nally, K. (2021). TNF-α synergises with IFN-γ to induce caspase-8-JAK1/2-STAT1-dependent death of intestinal epithelial cells. *Cell Death & Disease*, 12(10), 864. https://doi.org/10.1038/s41419-021-04151-3

89. Xavier, R. J., & Podolsky, D. K. (2007). Unravelling the pathogenesis of inflammatory bowel disease. *Nature, 448*, 427–434.

90. Zhang, Q., Pan, Y., Yan, R., Zeng, B., Wang, H., Zhang, X., Li, W., Wei, H., & Liu, Z. (2015). Commensal bacteria direct selective cargo sorting to promote symbiosis. *Nature Immunology*, 16, 918–926. https://doi.org/10.1038/ni.3233

91. Zhang, Y., Ma, S., Li, T., Tian, Y., Zhou, H., Wang, H., & Huang, L. (2023). ILC1-derived IFN-γ regulates macrophage activation in colon cancer. *Biology Direct*, 18(1), 56. https://doi.org/10.1186/s13062-023-00401-w. Erratum in: *Biology Direct*, 18(1), 83.

92. Zheng, Y., Valdez, P. A., Danilenko, D. M., Hu, Y., Sa, S. M., Gong, Q., Abbas, A. R., Modrusan, Z., Ghilardi, N., de Sauvage, F. J., & Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nature Medicine, 14*(3), 282-289. https://doi.org/10.1038/nm1720

5. VITAE

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EDUCATION

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ACCOMPLISHMENTS, ACTIVITIES, CONFERENCES ATTENDED

Vice President *Graduate Student Association for Biomedical, Georgia State University,* (September 2017 – August 2018)

Digestive Disease Research Day Conference- Stone Mountain, GA (September 2022)

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Preprint Manuscript

Johnson, AE., Denning, Timothy L. (2024) Modulatory Effects of IFN-γ and IL-22 on Inflammatory Signaling and Cellular Responses in Intestinal Epithelial Cells. bioRxiv https://doi.org/10.1101/2024.05.09.593373