The Dissertation Committee for Gabriela Uribe Certifies that this is the approved version of the following dissertation:

Protective Role of Mesenchymal MyD88 Signaling Under Homeostasis and Initiation of Inflammation in the Colon

Committee:

Iryna V. Pinchuk, Ph.D., Supervisor

Ellen J. Beswick, Ph.D.

Ashok K. Chopra, Ph.D.

Yingzi Cong, Ph.D.

Mark Hellmich, Ph.D.

Dean, Graduate School

Protective Role of Mesenchymal MyD88 Signaling Under Homeostasis and Initiation of Inflammation in the Colon

by

Gabriela Uribe, B.S.

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas Medical Branch

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas Medical Branch

July 2019

Dedication

This work is dedicated to my family and friends, who have inspired me to pursue my graduate studies. I am so grateful for their love and support that has allowed me to reach this milestone.

Acknowledgments

I would like to acknowledge my family and friends for being supportive of me throughout this journey.

To my committee members, for their contribution to my development as a researcher. Specifically, I would like to acknowledge Dr. Beswick for her support and active involvement in the design of my experiments during this process.

To Dr. Hellmich and Dr. Chao, for giving me the opportunity to be a TL-1 scholar and contributing to the development of my project.

To Dr. Singh, for giving me the opportunity to join the Cell Biology Graduate Program, playing a critical role in the initial development of my thesis, and for her support throughout my graduate studies.

I would like to give a special acknowledgement to Dr. Powell for making a significant contribution to the completion of my first manuscript, for his encouragement, and for playing a pivotal role as a co-mentor, which has contributed to my development as a researcher.

I would also like to give a special acknowledgement to Dr. Pinchuk for giving me the opportunity to pursue my graduate studies, forcing me to challenge myself, her guidance, and for being an influential graduate mentor.

Protective Role of Mesenchymal MyD88 Signaling Under Homeostasis and Initiation of Inflammation in the Colon

Publication No._____

Gabriela Uribe, Ph.D.

The University of Texas Medical Branch, 2019

Supervisor: Iryna V. Pinchuk, Ph.D.

Abstract Intestinal Mesenchymal stromal cells (fibroblasts, myofibroblasts and their progenitors) have recently emerged as key players in the interplay between the microbiota and professional immune cells under homeostasis and chronic inflammation. Initiation of microbial interactions with host cells, including stromal cells, requires myeloid differentiation factor 88 (MyD88). Overall, MyD88-dependent interactions between the microbiota and the host is reported to contribute to epithelial barrier restoration via repositioning of mesenchymal stromal cells producing COX-2/PGE_2 during injury. However, whether MyD88 signaling in mesenchymal cells is involved in this process is not known. Moreover, mesenchymal cell MyD88 signaling is involved in mucosal tolerance via suppression of type 1 adaptive immune responses. However, the impact of MyD88-dependent signaling within mesenchymal stromal cells on professional innate immune cells such as macrophages within the gut mucosa is poorly understood. Therefore, in this project I evaluate the hypothesis that MyD88-mediated signaling in mesenchymal stromal cells is required for the control over inflammatory responses in the colon. To test this hypothesis, I used primary human mesenchymal cells in culture, as well generated several mesenchymal stromal cell-specific MyD88-inducible knockout mice under homeostasis and in a model of acute colitis using DSS. Histological analysis was used to determine inflammatory and fibrotic changes in the colonic mucosa. The intracellular and cellular responses were analyzed by using RNAseq, qRT-PCR, ELISA, western blot, cytokine/chemokine multiplex array, flow cytometry, and confocal microscopy. In our first aim, I found that deletion of MyD88 within mesenchymal stromal cells *in vivo* resulted in inflammatory changes and increased fibrosis within the colonic mucosa as well as moderately aggravated DSS induced acute colitis. In addition to IBD-type dysbiotic changes of the fecal microbiota in mice lacking MyD88 within mesenchymal stromal cells, RNAseq analysis of the colonic mucosa from these mice revealed changes in pathways controlling epithelial barrier maintenance and innate and adaptive cell inflammatory responses in DSS-treated and non-treated animals. In order to understand how stromal cell-intrinsic MyD88 signaling activated by the microbiota contributes to the regulation of intestinal homeostasis and how it is disrupted during the immunopathogenesis of IBD, we used *Lactobacillus rhamnosus* GG (LbGG) as a representative species of normal beneficial gut microbiota. I demonstrated that mesenchymal stromal cells isolated from the normal human colonic mucosa responded to LbGG with activation of the eicosanoid pathway resulting in increased expression of COX-2 mediated PGE_2. Similar observation were made *in vivo*, using a murine model. I also show that in culture and *in vivo* this process requires MyD88 signaling within mesenchymal stromal cells. Taken together, these data suggest that mesenchymal stromal cells are among the major contributors to the increase of COX-2-mediated PGE_2 in response to the normal microbiota, contributing to the maintenance of mucosal homeostasis in the colon via MyD88. In experiments performed in my second aim, the inflammatory changes observed in our initial RNAseq analysis were associated with increased infiltration of $F4/80^+CD11b^+$ macrophages. Increase in $CX3CR1^{high}CCR2^+$ macrophages producing TNF-α was also observed and depletion of the macrophages with clondronate resulted in a decrease in total TNF-α levels within the colonic mucosa. This suggests that there is increased chemotaxis of inflammatory macrophages to the colonic mucosa. In conclusion, my data from my work suggest that MyD88 signaling within mesenchymal stromal cells contributes to colonic mucosal homeostasis through the production of PGE_2 and the suppression of the influx of TNF-α and IL-6 producing inflammatory macrophages.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

5-LO	5-lypoxygenase
AA	Arachidonic Acid
AJ	Adherens junctions
BM	Bone marrow
CD	Crohn's Disease
CMFs	Colonic CD90+ (myo) fibroblasts
COX-2	Cyclooxygenase 2
cPLA2	Cytosolic phospholipase A2
DSS	Dextran Sulfate Sodium
EB	Epithelial Barrier
ECM	Extracellular matrix
ER-TR7	anti-reticular fibroblasts and reticular fibres antibody
Fib-MyD88 KO	Tamoxifen-inducible MyD88 floxed mice; specific to Col1α2+ cells.
GI	Gastrointestinal
i.p.	intraperitoneal injection
IEC	Intestinal Epithelial Cells
IFN-γ	Interferon gamma
IL-1	interleukin 1 receptors
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-6	Interleukin-6
ILC	Innate lymphoid cells
IPA	Ingenuity pathway analysis
KO	Knockout
LbGG	Lactobacillus rhamnosus GG

MC	Micro-colitis
MF-MyD88 KO	Tamoxifen-inducible MyD88 floxed mice; specific to α-SMA+ cells
MFs	Myofibroblasts and fibroblasts
MLN	Mesenteric lymph node
MRS	De Man, Rogosa and Sharpe
MSC	Mesenchymal stem cells
MyD88	Myeloid differentiation factor 88
PDGFRα	platelet-derived growth factor receptor A
PD-L1	Programed death ligand 1
PGE2	Prostaglandin E2
Th	T-Helper
TJ	Tight junctions
TLRs	Toll-like receptors
TMX	Tamoxifen
TNF- α	Tumor necrosis factor- α
Treg	Regulatory T cells.
UC	Ulcerative Colitis
WB	Western blot
α-SMA	smooth muscle actin

CHAPTER 1. GENERAL INTRODUCTION

Objective of the dissertation

My dissertation project is focused on the role of MyD88 signaling in colonic mucosal stromal cells otherwise known as (myo-/fibroblasts, MF) in the regulation of intestinal homeostasis and how it is disrupted during inflammation relevant to the immunopathogenesis of Inflammatory Bowel Disease (IBD). In Chapter I, I will introduce several common concepts relevant to the field of my study, current knowledge in the field, and identify the major gap in knowledge. In chapter II, using Lactobacillus GG (LbGG) as a model organism for the normal colonic flora, I describe the role of stromal cell-intrinsic MyD88 signaling in the maintenance of colonic homeostasis through the interaction with the gut microbiota and subsequent influence on inflammatory processes. Chapter III focuses on the influence of mesenchymal stromal cell MyD88 signaling to the maintenance of homeostasis and during the inflammation relevant to the immunopathogenesis of IBD through the interaction with macrophages. In chapter IV, I will discuss the overall biological and translational significance of the obtained results, and provide conclusions and future directions for my project. Briefly, my work demonstrates that mesenchymal stromal cell-intrinsic MyD88 signaling participates in the maintenance of the epithelial barrier, gut microbiota populations, and in the suppression of inflammatory responses attributed by inflammatory macrophages in both in homeostasis and IBD-relevant colitis animal model.

Host-microbiota interactions and intestinal homeostasis.

The gastrointestinal tract is inhabited by a large number of microorganisms including viruses, fungi, and over 1000 species of bacterial that are collectively called the microbiome[1]. In the healthy human gut bacteriome the Firmicutes phylum is more prevalent over Bacteroidetes. However, during dysbiosis this balance is inversed[2] Diversity in the microbial composition exists between individuals, but the fundamental role in of the microbiota in nutrition, metabolism, and resilience to pathogens is conserved because of the overall metagenomic expression of the sum of the microbiota[3].

Interplay between normal gut microbiota and the host is not only essential for the digestion and absorption, but also critical to the development of the innate and adaptive immunity, especially in the context of the mucosal tolerance, which is critical to homeostasis in the host.[4] These homeostatic host-microbe interactions have been shown to be implicated in both the promotion of beneficial microbiota growth and inhibition of colonization of the gut by pathogenic and opportunistic bacteria.[4-6] It is clearly established that complex interactions between the microbiota and the host innate immune cells, including non-professional immune and adaptive immune system, cooperate to maintain intestinal homeostasis. Although, most of the knowledge about the specific mechanism(s) that drive these interactions are drawn from the exploration of the immune responses to pathogenic microbes in the inflammatory settings.[6] Thus, there is a need to determine the mechanisms of how innate cells engaged in this homeostatic dialogue with commensal microbiota to prevent maintain homeostasis and how dysbiosis contribute to the dysregulation of the innate immune cell functions during the onset/progression of the inflammation in the gut.

Inflammatory Bowel Disease epidemiology, pathophysiology, and treatment.

Inflammatory bowel disease (IBD) is a complex and chronic disease associated with a dysregulated immune responses to environmental triggers (in particular dysbiotic microbiota) in the genetically susceptible host. IBD is a chronic incurable disease that primarily effects people in developed countries, with 3 million children having been affected in the United States [7-9]. The two major types of IBD, Crohn's disease (CD) and ulcerative colitis (UC), represent distinct pathophysiological entities. In particular, CD is characterized by discontinuous transmural ulceration and fibrosis of the gastrointestinal (GI) tract, and is associated with frequent relapsing flares that result in diarrhea, abdominal pain, and rectal bleeding.[10] In contrast to CD, inflammatory changes in UC are more restricted to the colonic mucosa, while CD inflammation may affect all layers of the intestinal wall.[10, 11] Up to 50% of patients with CD experience complications associated with continuous ulceration, such as: fistula, abscess, and fibrostenoic strictures [12, 13]. As a result of these complications, most patients with CD require surgery at least once in their lifetime [14]. More recently, UC was also associated with mild fibrosis that is linked to the severity and chronicity of inflammation [15].

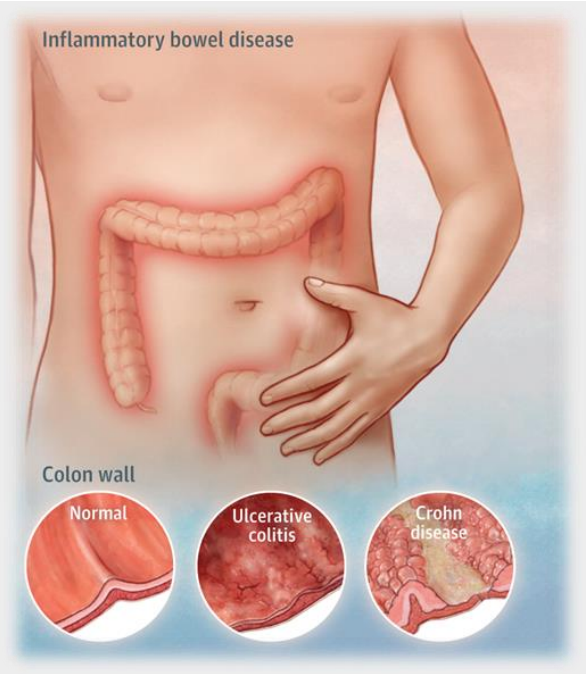

Figure 1. IBD is a chronic inflammatory disease that primarily affect the gastrointestinal tract. The 2 major forms of IBD are Crohn's Disease and Ulcerative Colitis. Adopted from *Friedrich MJ. Inflammatory Bowel Disease Goes Global. JAMA. 2018;319(7):648. doi:10.1001/jama.2018.0365*

However, the development of fibrosis in ulcerative colitis has remained largely unexplored [16]. While the full mechanism of fibrosis is far from understood, it is clearly established that IBD fibrosis is characterized by an exaggerated response and by accumulation of collagen-rich but abnormally reorganized extracellular matrix (ECM).[17]

Another chronic intestinal inflammatory disease known as microscopic colitis (MC) is commonly under-recognized in clinical practice [18]. MC is characterized clinically by chronic non-bloody diarrhea and histologically by two features- collagenous and lymphocytic colitis (Figure 2)[19]. However, recent studies have identified a significant genetic overlap of MC with CD, suggesting a common underlying mechanism for these diseases. Thus, both subtypes of MC were recently proposed to be included as a subcategories of IBD. Finally, it has been observed that recurrent MC may precede the development of CD or UC. Thus, presentation of recurrent MC symptoms leads to the evaluation for other types of IBD. However, very little is known about the etiology and pathophysiology of the MC and how it may progress to UC or CD.

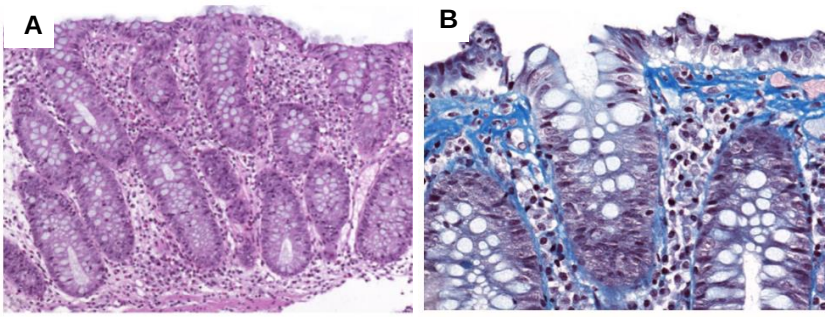

Figure 2. Microscopic colitis is characterized histologically by two features- collagenous and lymphocytic colitis. (A,) Lymphocytic colitis with a significantly increased number of surface intraepithelial lymphocytes (IELs) and no crypt architectural distortion.(B) Masson trichrome immunostaining highlight the collagen band and illustrate the characteristic jagged appearance at the deeper border. Adapted from *C. Langer. Histology of microscopic colitis-review with a practical approach for pathologists. Histopathology. 2015 Apr;66(5):613-26. doi: 10.1111/his.12592.*

Since there is no cure for IBD, the aim of current therapeutics are not to cure the disease, but to achieve disease remission in order to improve quality of life. Current therapeutics involve the use of anti-inflammatory aminosalicylates, corticosteroids, immunosuppressants such as 6-mercaptopurine or cyclosporine, biological monoclonal antibodies against TNF-alpha, and partial or total surgical resection of the intestine in cases where these therapeutics are unsuccessful. [20, 21] Evaluation of the changes in the IBD intestine's barrier function, gut microbiota, matrix remodeling, macrophage and lymphocyte activation, homing and retention may result in the discovery of potentially novel therapeutic [22]. However, despite the improved efficacy of novel standard of care drugs, such as biologics, to control inflammation, little of

progress has been made to prevent progression of intestinal inflammation and subsequent fibrosis in IBD.[17] In summary, many challenges remain in understanding the mechanisms that contribute to the initiation and chronicity of IBD and its associated diseases. This gap in knowledge precludes the development of curable therapeutic strategy against these devastating diseases.

IBD Etiology: role of microbiota and innate immune system in the initiation or the disease.

Although the etiology of IBD remains elusive, it is established that a combination of environmental factors, result in the alteration of the gut microbiota, mucosal barrier defects, and leads to overt inflammatory activation of innate and adaptive immune cells in genetically susceptible individuals[23]. In IBD, dysregulated permeability of the intestinal epithelial barrier (EB) in a hyper-immune reactive environment sets the stage for immunopathogenesis. Intestinal permeability is regulated at the luminal face by linkage of intestinal epithelial cells (IEC) via tight junctions (TJ) and adherens junctions (AJ) (Fig.3)[24, 25]. Changes in the diversity of the microbiota (a.k.a. dysbiosis) contributes to immunopathogenesis of IBD[26]. The microbiota can help preserve intestinal EB integrity by promoting the maintenance of TJ, promoting epithelial repair, and by forming the stem cell

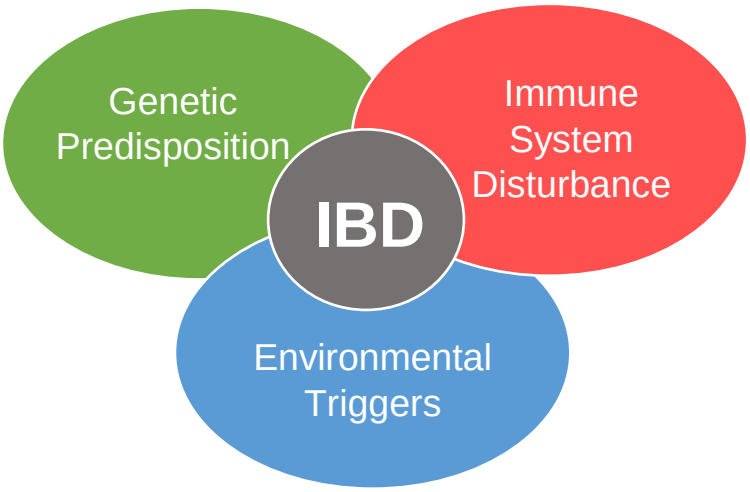

Figure 3. **IBD is a chronic multifactorial disease**. Genetic predisposition, environmental triggers, microbiota, and immune responses contribute to the disease pathogenesis. Adapted from *Loddo, I., & Romano, C. (2015). Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. Frontiers in immunology, 6, 551. doi:10.3389/fimmu.2015.00551*

niche that regulates turnover of enterocytes[24-26]. The chronic mucosal inflammation in both CD and UC result in hyperactivation of innate and adaptive immune cells, which produce high levels of pro-inflammatory cytokines including, but not limited to, TNF-α, IL-1β, IL-6, as well type 17 cytokine IL-17A.[27-29] Additionally, increase in the type 1 cytokine IFN-γ produced mostly by $CD4^+$ T helper (Th) and innate lymphoid cells (ILCs) was observed in CD. In contrast, increase in IL-5 and IL-13 produced by Th2, NKT, NK and ILCs was observed in UC. This burst of inflammatory cyotkines results in intestinal tissue damage.[27-29] Furthermore, IBD-associated susceptibility cytokine loci are involved in the regulation, development and function of intestinal Th and regulatory T cell (Treg) subsets. [27-29]

The mechanisms responsible for dysregulation of adaptive immune cell responses, and in particular $CD4^+$ T cells in IBD, remain unknown. Innate immune cells are also among the first cells initiating the interaction with the microbiome in the gastrointestinal mucosa[30]. Therefore, there is an emerging need to understand how these cells contibute to the hyperactivation of the adaptive immunity and overall onset and progression fo the iflammation in IBD.

Role of Macrophage in the homeostasis and IBD type inflammation.

Among the gut mucosal innate immune cells, macrophages constitute a large portion of mononuclear phagocytic cells that are critical to pathogen recognition and elimination.[31] The optimal set of markers for these cells in both the human and murine gut mucosa remains debatable. However, it is clear that in murine intestinal mucosal and mesenteric lymph nodes (MLN) macrophages can be identified by the expression of F4/80, CD64, and CD11b markers [3, 4] and upon maturation, begin to express increased levels of the homing chemokine receptor CX3CR1 [5][32, 33] In the human gut, these cells can also be identified by their expression of pan-myeloid cell markers, including CD33, CD14, and CD13. Additionally, humans and mice macrophages preserve positivity for CD64. When

activated in both species, macrophages express high levels the antigen presenting molecule MHC II and co-receptor molecules necessary for antigen presentation such as CD68, CD80 ,and CD86[31-33]. Macrophages are suggested to be able to discriminate between pathogens from commensal microorganisms through pathogen recognition of microbial molecules [31]. As a results of these interactions, these cells respond to the acute infection within the burst of inflammatory responses such as increase in NO and IL-12, contributing to the clearance of the pathogens and simultaneously mounting adaptive immune responses via antigen presentation process [31]. In contrast, interaction of these cells with commensal microbiome often results in in tolerogenic responses via mechanism involving the production of anti-inflammatory cytokines [25][32, 33]

Classically, macrophages have been classified into one of two major subtypes, M1 and M2, based on the type of expression of surface receptor and co-receptors as well the type of secreted inflammatory mediators. M1 macrophages produce inflammatory cytokines to support other immune cell functions, while M2 produce IL-10 to inhibit them [34]. It was initially reported that M1 macrophages are typically activated by interferon gamma derived from Th1 cells, and secrete pro-inflammatory cytokines and chemokines. While, the alternatively activated M2 macrophages, are anti-inflammatory and crucial to wound healing. M2 macrophages are defined by the ability to produce molecules implicated in tissue repair, fibrosis, and dampening of inflammatory responses [33, 34]. However, over the last five years it has been clearly shown that gut-resident macrophages do not fully fit into the M1-M2 paradigm and often portray an intermediated function between M1 and M2, demonstrating overlap in the cytokines produced. For instance, a subset has recently been described that produces IL-10, as M2 macrophages do, but in contrast to M2 macrophages also produce pro-inflammatory IL-1β, IL-6 and TNF-α [33].

Additionally, macrophages in the gut are also classified as resident and *de novo* derived from peripheral blood circulating monocytes. In the steady state mucosa, normal gut resident macrophages function as sentinels, contributing to the clearance of apoptotic or

senescent cells and remodeling of tissue [33]. These resident macrophages are reported to produce a variety of cytokines, including IL-10, that helps maintain tissue homeostasis [33]. Simultaneously, these cells are critical sensors of the pathogens and shown to be highly phagocytic and are actively bactericidal. Stimulation of Toll-like receptors (TLRs) on the resident macrophages by the pathogenic microbiota, have been shown to stimulate the production of inflammatory IL-1β [29][33]. In contrast to the inflammatory macrophages freshly differentiated from peripheral blood monocytes, resident intestinal macrophages do not elicit a full burst of inflammatory responses, such as production of TNFα, IL-1, IL-6, or other inflammatory cytokines and chemokines upon exposure to the pathogenic bacteria and their products [32, 33]. Proinflammatory macrophages, including those differentiated from monocytes, are essential to resolving pathogenic bacterial infections through the production of cytokines [13, 31–33]. While mediating bactericidal function, macrophages also contribute to mucosal tissue repair of epithelial damage through the production of IL-10, which is imperative to resolving intestinal inflammation [35, 36]. Accumulation of $CD14^{hi}$ and $Ly6C^{hi}$ macrophages has been shown in the mucosa of IBD patients and murine models of colitis, respectively [31-33]. These inflammatory macrophages are suggested to be critical for the initiation of inflammation in IBD. It is also believed that altered recognition of commensal gut microbiota by macrophage is a key contributor to intestinal inflammation and ultimately give rise to diseases such as IBD [30]. However, mechanisms regulating the influx of monocytes to become *de novo* resident macrophages within the gut mucosa is not fully understood.

Mesenchymal stromal cells are critical contributors to homeostasis and IBD.

Myofibroblasts and fibroblasts (MFs) in colonic mucosa: tissue architecture and major markers.

The colonic mucosa is made up of 3 layers: the epithelium, the *lamina propria* and the *muscularis propria* [37]. The *lamina propria* contains at its most external extent a layer of smooth muscle cells, 2-3 layers thick which is called the *muscularis mucosae*. This the part

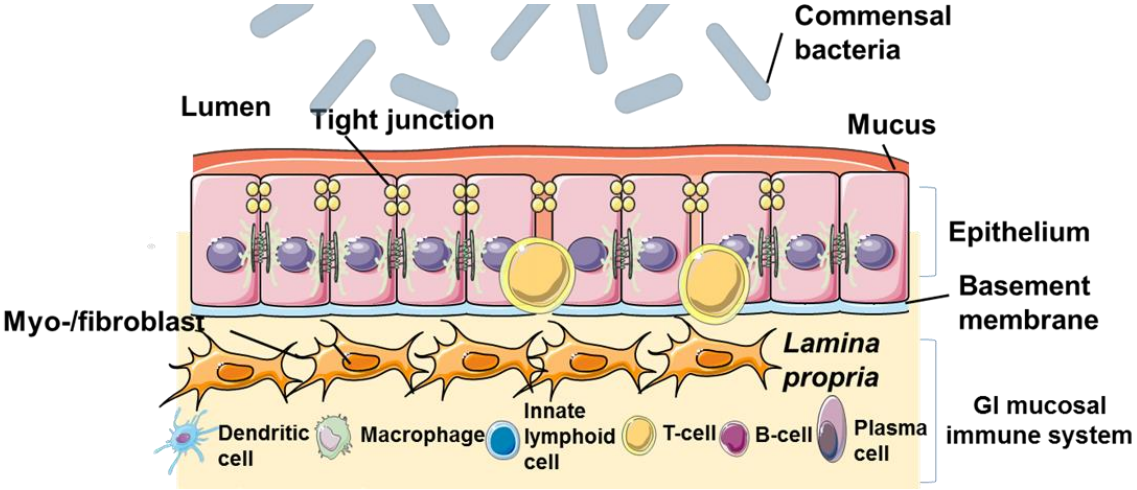

Figure 4. The epithelial cells and the myo-/fibroblast in the *lamina propria* are innate cells linking the microbiota and adaptive immune responses. Schematic demonstrating the cells within the colonic mucosa. The intestinal epithelium provides a protective barrier against the luminal intestinal microbiota from the underlying lamina propria (LP). Intestinal epithelial cells (IECs) are comprised of distinct subpopulations. Among these subpopulations, goblet cells, which secrete mucins and peptidoglycan, form a mucus layer overlaying IECs that provides a chemical and physical barrier against these microorganisms. Underlying the IECs, the LP contains plasma cells, macrophages, dendritic cells, and myo-/fibroblasts. Under homeostasis, these cells have a limited expression of inflammatory cytokines.

of the *lamina propria*. The *muscularis propria* is the muscular layer of the gut and is made up of interconnected bands of circular and longitudinal smooth muscle each several cell layers thick [37].Cells of mesenchymal origin, including myofibroblasts, fibroblasts, and stromal mesenchymal progenitor (stem) cells are major elements of the intestinal *lamina propria,* making up 30% of the mucosal *lamina propria* [38]. (Figure 4). In humans, but not mice, most cells of mesenchymal lineage are identified by the expression of extracellular glycoprotein CD90, also referred to as Thy-1 [39]. In the intestinal mucosa, CD90 marks fibroblasts, myofibroblasts and the mesenchymal progenitors [39] . Within the human colonic mucosa these cells also reported to express type 3 intermediate filament protein subtypes of mesenchymal cells vimentin but are negative for desmin [39].More recently platelet-derived growth factor receptor A (PDGFRα a.k.a CD140a), has emerged in mice and humans as an important marker for these cells [40, 41]. It has been speculated that upon injury, intestinal

fibroblasts become activated and subsequently differentiate into myofibroblasts. In the colon, myofibroblasts can be distinguished by the expression of intracellular cytoskeleton microfilament protein alpha smooth muscle actin (α-SMA)[42]. Moreover, in humans and mice, anti-reticular fibroblasts and reticular fibres antibody [ER-TR7], which have been shown to mark thymic stromal cells, has also been identified as a potential marker for colonic myofibroblasts *in situ* [43, 44]. Our team was able to use variations of these markers to further characterize the activity of different cellular phenotypes in their contribution to homeostasis and during injury in the context of the gastro-intestinal tract [38, 45-48].

Role of MF in the Epithelial Barrier (EB) Maintenance.

MF and their progenitors play a critical role in the promotion of EB restoration and maintenance after intestinal injury6 [49]. MFs promote resolution of inflammatory activity accompanied with balanced repair processes [50]. MFs are among the major contributors to wound repair and function through the secretion of prostaglandins, secreting enzymes that break down fibrin assembly, secreting extracellular matrix (ECM), contracting the wound, and building collagen structures to support the cells associated with effective wound repair[51]. During epithelial restitution, fibroblasts are activated (differentiated) into myofibroblasts expressing α-SMA at the inflammation/injury site by professional immune cells producing TGF-β1 [50]. Activated MFs are demonstrated to be critical to the deposition of extracellular matrix (ECM) molecules such as collagen and tenascin C, and promote mucosal repair by appropriately adjusting the production and degradation of the ECM [50]. In addition, myofibroblasts produce growth factors (e.g., HGF), which induces epithelial cell proliferation [50]. The transient appearance of activated mesenchymal cells is a feature of normal wound healing, but the persistence of these cells is associated with tissue fibrosis. Recent studies suggest that mesenchymal cells derived from bone marrow (BM) stem cells play a crucial role in intestinal repair and IBD fibrosis [52]. Despite our advance in the

understanding critical role of the MF in the epithelial barrier restoration and fibrosis, little is known how microbiota influence these processes.

MFs are critical source of COX-2 dependent PGEs under homeostasis and inflammation.

Several intestinal mucosal cells are reported to produce COX-2-dependent PGE_2 during injury, inflammation, and cancer [53]. In contrast, cells of mesenchymal origin are known to produce PGE_2, not only during inflammation and cancer but also in GI homeostasis[39, 54]. Among such cells, colonic $CD90^+$ (myo) fibroblasts (CMFs) are abundant innate immune cells in the normal GI mucosa that have recently emerged as key contributors to GI mucosal homeostasis[39, 42, 48, 55]. Following epithelial injury, intestinal (myo) fibroblasts are reported to sense inflammatory signals and activate COX-2-dependent PGE_2 production, contributing to the regeneration of the epithelium [56]. Repositioning of COX-2-expressing mesenchymal stem cells (MSC)-like cells in the intestinal mucosa has been shown to have a radioprotective effect and contribute to wound healing [57, 58]. Our laboratory previously reported that human normal (N)-MFs express IL-1R and in response to IL-1, express increased levels of COX-2 protein, resulting in higher levels of PGE_2 [59].

It is important to note that the GI tract, and in particular the colon, is highly populated by the microbiota, which is important to the maintenance of homeostasis. The microbiota also strongly influences immune responses to injury, justifying current interest in microbiota-based probiotic therapies for several GI pathologies[60]. However, little is known about how MF-restricted COX-2/PGE_2 is regulated by the normal gut microbiota under homeostasis.

MFs serves as innate immune cells in the normal and IBD mucosal lamina propria.

Our laboratory published that intestinal MFs (MFs) are important innate immune cells, and while they may serve as non-professional APCs,[38] are among the major suppressor of the inflammatory responses in the normal mucosal *lamina propria* [61](Figure 5). MFs have been shown to participate in tolerogenic responses through expression of B7 suppressor molecules, PD-L1 and PD-L2 [61]. Our laboratory has demonstrated that CMFs are active contributors to both innate and adaptive immune responses during homeostasis, chronic inflammation, and cancer[48, 62, 63] Using primary cell culture *in situ* and *in vivo* experimental approach, our team reported that normal myo-/fibroblasts play an immunosuppressive role contributing to mucosal tolerance [39, 48, 55, 61]. An increase in an activated "myofibroblast" phenotype was observed in CD by our laboratory and others [39, 64]. When compared to normal controls, these cells show a decrease expression of the Th1/Th17 suppressive molecule PD-L1 in CD, while expression of this molecule was increase on MF in UC [64]. Our laboratory also demonstrated that MFs function is changed in chronic IBD and support the dysregulation of Th1 immune responses (Figure 5).[64] These data differentially support upregulation of pathogenic T cell responses (Th1 in Crohn's disease and Th2 in ulcerative colitis) in co-culture [64]

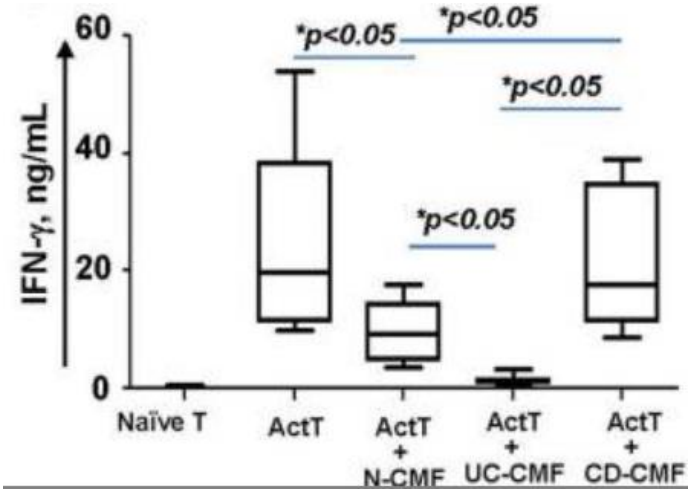

Figure 5. UC-CMFs strongly suppress Th1 type responses (IFN-γ) in activated CD4+ T cells, while suppression by CD-CMF is reduced. IFN-γ production was analyzed using singleplex cytokine analysis in actiavated CD4+ T cells co-cultured with normal, UC, and CD -CMFs. *Beswick EJ, Grim C, Singh A et al Expression of Programmed Death-Ligand 1 by Human Colonic CD90+ Stromal Cells Differs Between Ulcerative Colitis and Crohn's Disease and Determines Their Capacity to Suppress Th1 Cells. Front Immunol. 2018;9:1125.doi:10.3389/fimmu.2018.01125*

Interplay between cells of mesenchymal origin and macrophages.

Newer studies suggest that MSCs can induce immunomodulatory M2-like macrophages in vitro that can inhibit T cell and NK cell function and induce Tregs ([65-69]. In general, the main MSC-derived molecule that promotes the M2 activation state is PGE_2 ([65]. Bone marrow derived mesenchymal cells contribute to macrophage differentiation via PGE_2 [70]. Additionally, mesenchymal cells are involved in the recruitment of monocytes in a systemic salmonella infection model via CCL2 in the liver and spleen [71]. However, the role of by mesenchymal stromal cells in the regulation of inflammatory responses by macrophages in the colonic mucosa is not known.

MyD88 is required for activating TLRs and IL-1/IL-1R signaling.

Myeloid differentiation factor-88 (MyD88) functions as a universal adapter protein to regulate the signaling of most TLRs and interleukin 1 receptors (IL-1R)[72]. MyD88 acts as a central hub in inflammatory responses and can induce signaling from several receptors[73]. MyD88 signaling can lead to the production of pro- or anti-inflammatory cytokines [73]. These cytokines can further influence other immune cells and accordingly dictate the tone of an immune response [74]. For example, bacterial stimulation of the TLR/MyD88 pathway modulates intestinal homeostasis [75] and plays a crucial role in recognition and response to microbial pathogens to maintain the epithelial barrier integrity in the intestinal tract [72]. MyD88 signaling pathway also plays a critical role for host defense and survival during infection [76]. Additionally, MyD88 signaling in the gut plays an important protective role in both acute and chronic models of colitis mimicking IBD, suggesting its role in this disease [77] [78]. Signaling of the intestinal microbiota through Toll-like receptors (TLRs) is critical to maintenance of mucosal homeostasis and to the development/progression of IBD [48, 79]. Changes in the diversity of the microbiota (a.k.a. dysbiosis) contributes to immunopathogenesis of IBD [25, 26]. In IBD, disruption of the intestinal EB, along with increased TLR4 and 5 signaling, leads to the increased influx of immune cells to the site of mucosal inflammation[26]. In response to

microbial dysbiosis, mucosal cells produce higher levels of inflammatory cytokines, contributing to ulceration and subsequent fibrosis[1].

It has been suggested that PGE_2 signaling may be an important target to enhance Th17 actions and improve intestinal host defense in a *Cittrobacter rodentium* model of infection [80]. It has also been shown that stability of microbiota populations are influenced by COX-2 activity, and when this homeostatic process is interrupted, mice have dramatically increased mortality and the intestinal pathology associated with Clostridium difficile infection [81]. Furthermore, signaling through TLR2 contribute to the protection against radiation injury in the small intestine of mice through repositioning of COX-2 expressing cells[58].

MyD88 in innate immune signaling

MyD88 signaling through innate immune cells is critically involved in microbial recognition, induction of antimicrobial products, and modulation of the adaptive immune response in the colonic mucosa [82-84]. The consequences of MyD88 deficiency appears to be both pathogen and tissue-specific, suggesting substantial complexity in innate defenses[85]. Cellular compartmentalization of MyD88 signals in the intestines is essential for the maintenance of homeostasis and to prevent deleterious inflammatory responses [86].

MyD88 signaling in IECs appears to be crucial for maintenance of gut homeostasis, since specific deletion of MyD88 in IECs results in compromised antibacterial immunity[87]. In particular, MyD88 signaling in intestinal epithelial cells (IEC) has been shown to be crucial for maintenance of gut homeostasis[87]. While, MyD88 signaling in both dendritic cells and IEC is essential to induce a full spectrum of host responses upon intestinal infection with *Citrobacter rodentium*[88]. Alternatively, hematopoietic, and not epithelial cells, transmit the MyD88-dependent response to *Helicobacter hepaticus* in a spontaneous chronic colitis model [86].

MyD88 expression in the mononuclear phagocyte (MNP) compartment, which includes macrophages, has been shown to be required for colitis development in the absence of IL-10 [89]. However, MyD88 signaling in macrophages has been shown to facilitate the phagocytosis of different pathogens [90] and critical for the resolution of infections [91, 92]. During acute intestinal injury in mice treated with DSS, MyD88 expression by myeloid cells were shown to be critical for repair of the EB [82]. Although MyD88 in macrophages has been shown to be critical for both intestinal homeostasis and response to injury models of IBD, It has also been noted that, MyD88-dependent signaling from a non-myeloid cell type, but not defined cell type, is required to maintain colonic homeostasis [82]. Our laboratory also show that in the normal colonic mucosa signaling through TLR-MyD88 is required to sustain type 1 inflammatory responses in culture and *in vivo* [48] (Figure 6).

Role of MyD88 dependent signaling within MFs in the homeostasis and IBD.

While it is clearly established is that MF critically contribute to the EB repair and maintenance of the tolerogenic immune responses within intestinal mucosa, the interplay between bone marrow-derived mesenchymal cells (BMMSCs) with the microbiota is suggested to play a critical role in the resolution of inflammatory processes that contribute to wound healing. In the dextran sulfate sodium-induced experimental colitis mice, the normal microbiota is required to maintain immunomodulatory properties of BMMSCs through induction of activated T-cell apoptosis and cytokine secretion, whereas germ free-derived BMMSCs lose the capacity to ameliorate disease phenotypes.[93] Work by Brown SL *et al*. in murine models of acute intestinal injury demonstrated that Myd88 upstream of COX-2 dependent PGE_2 is required for the repositioning of a subset of the COX-2-expressing mesenchymal stromal cells contributing to the epithelial repair through stimulation of the

epithelial progenitor cells.[57] It has been also shown that expression of the Igf2bp1, a regulator of Ptgs2 gene expression in colonic mesenchymal progenitor cells, requires MyD88 signaling in mice.[94] Innate sensing through mesenchymal TLR4/MyD88 has been shown to promote intestinal tumorigenesis in an animal model of colon cancer.[95] Our laboratory also shows that in the normal colonic mucosa signaling through TLR-MyD88 is required to sustain type 1 inflammatory responses in culture and in vivo [48](Figure 6).However, the role of MF restricted MyD88 signaling within the colonic mucosa remains largerly unknown in both homeostasis and IBD type inflammation.

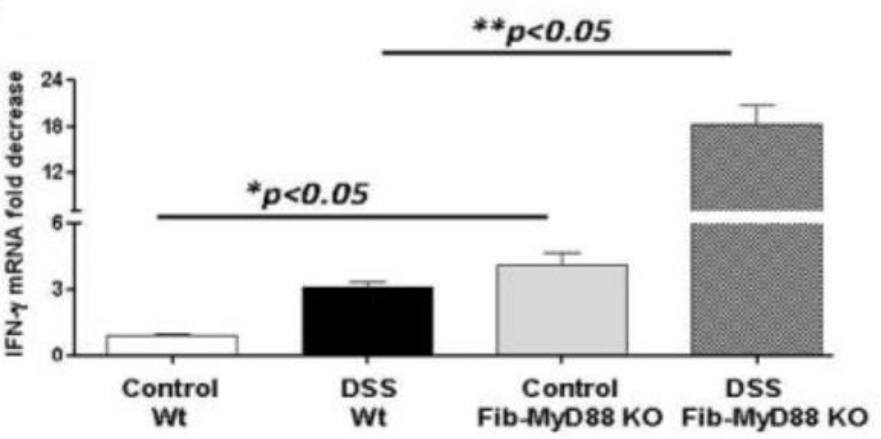

Figure 6. IFN-γ expression in the colonic mucosa is increased when Fib- MyD88 signaling is disrupted. IFN-γ mRNA level in colonic mucosa of mice lacking MyD88 in stromal cells (Fib-MyD88) treated with or without DSS was determined using real time RT-PCR**. I**FN- γ mRNA was normalized to β-actin. *Beswick EJ, Johnson JR, Saada JI, et al. TLR4 activation enhances the PD-L1-mediated tolerogenic capacity of colonic CD90+ stromal cells. J Immunol. 2014;193(5):2218–2229. doi:10.4049/iimmunol.1203441*

CHAPTER 2. CONDITIONAL DELETION OF MYD88 IN MESENCHYMAL STROMAL CELLS REVEALS ITS CRITICAL ROLE IN THE MAINTENANCE OF THE EPITHELIAL BARRIER VIA PGE2 SECRETION AND ITS DECREASED SUSCEPTIBILITY TO DSS-INDUCED COLITIS

Modified in part from:

***Lactobacillus rhamnosus* GG increases cyclooxygenase-2 expression and prostaglandin E2 secretion in colonic myofibroblasts via a MyD88-dependent mechanism during homeostasis.**

Uribe G, Villéger R, Bressollier P, Dillard RN, Worthley DL, Wang TC, Powell DW, Urdaci MC, Pinchuk IV. *Cellular Microbiology*. 2018; 20:e12871.

https://doi.org/10.1111/cmi.12871

Published: June 19th, 2018

Preliminary data, objective and working hypothesis

Myo-/fibroblasts (MFs) are abundant innate immune cells in the gut mucosal *lamina propria* located beneath the epithelial basement membrane [96]. It has been postulated that under homeostasis, normal gut microbiota promote EB integrity [97, 98]. Our lab previously published that in humans and mice MFs likely interact with the microbiota, because they express functionally active TLRs[99] and IL-1R[100]. We have also demonstrated that TLRs modulate MF-mediated regulation of Type 1 immune responses via MyD88 in the normal colonic mucosa [48].

Mucosal injury and fibrosis are major issues impeding the efficiency of current IBD therapy. Cells of mesenchymal origin, and in particular MFs, are critical players during injury[101]. Dysregulation of MFs in response to injury is believed to contribute to fibrosis in IBD. Dysbiosis in the gut microbiota is also shown to be among the key components contributing IBD[102, 103]. Increases in MyD88 dependent TLR4 and 5, as well IL-1 mediated signaling were observed in IBD[104]. While the importance of these signals in professional immune cells during immunopathogenesis of IBD has been demonstrated, their role in MF-mediating contribution to injury in IBD is unknown.

Our lab has successfully used several models of colitis that mimics processes relevant to IBD. Our lab also recently reported, using *in situ* and *ex vivo* analysis of human colonic mucosa, that human MFs may be local negative regulators of pro-inflammatory immune responses[48, 55, 61] and that CD MFs appear to switch from an immunosuppressive function to a pro-inflammatory function[62].Thus, the objective of the study described in this chapter is to define the in vivo contribution of MF-restricted MyD88 signaling to the regulation of intestinal homeostasis and during injury using recently developed fibroblast specific gene conditional knockout animals. In particular, I proposed the working hypothesis that MyD88 dependent signaling in mesenchymal stromal cells is involved in EB maintenance and control over inflammation under homeostasis and IBD-type colitis.

Results

Deletion of Myd88 within stromal cells increases inflammatory responses and aggravates mucosal damage in DSS colitis

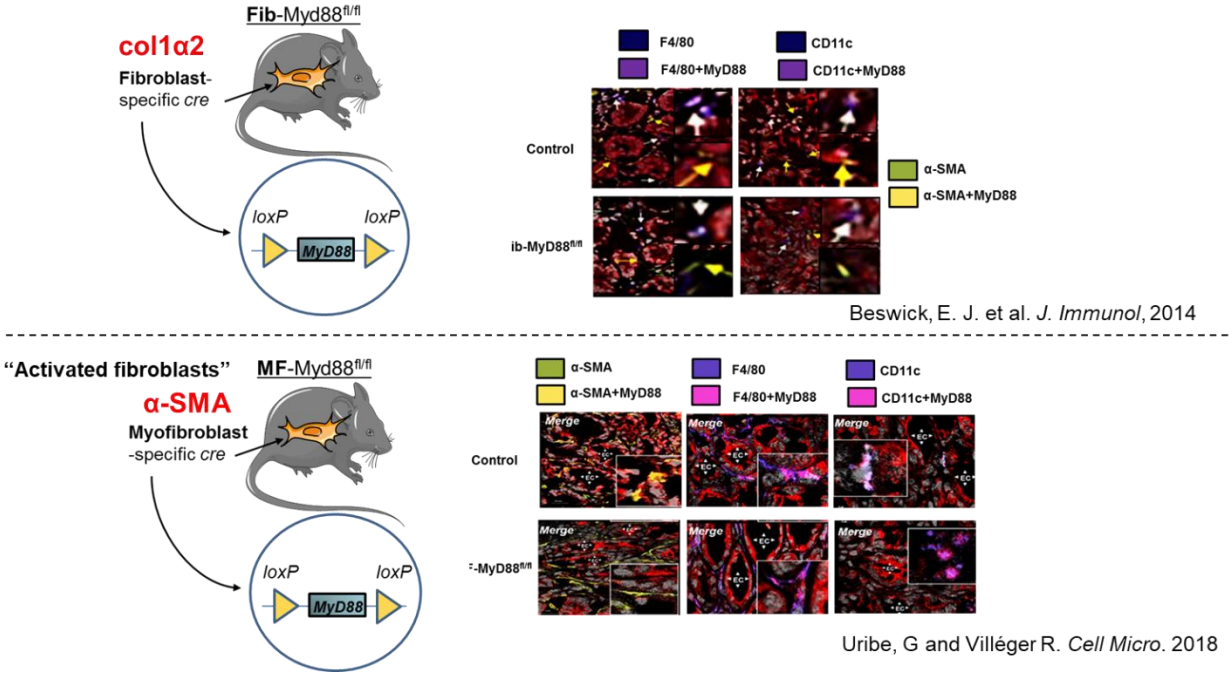

Figure 7. MyD88 was deleted within the α-SMA^{+}MFs in both Fib-MyD88$^{fl/fl}$ (col1α 2-Cre) and MF-MyD88$^{fl/fl}$ (α-SMA-Cre) mice, but retained in other innate immune cells within the lamina propria. Murine colonic mucosal sections of Fib-MyD88$^{fl/fl}$, used in all in vivo experiments throughout my dissertation (excluding *Lactobacillus rhamnosus* GG in vivo treatment in which we used α-SMA**-Cre-**MF-MyD88$^{fl/fl}$)were stained with anti-MyD88 mAbs (seen in red), -α-SMA mAbs (clone IA4)(seen in green), anti-murine CD11c mAbs (clone N418)(seen in blue), and/or anti-murine F4/80 mAbs (clone BM8)(seen in blue). Subsequent confocal analysis demonstrated that while MyD88 expression was abrogated in KO mice, MyD88 expression in F480^{+} and Cd11c^{+} cells were retained.

To examine the pathophysiological role of mesenchymal stromal cell-specific MyD88 in the disease pathogenesis of the Dextran Sodium Sulfate (DSS) model of intestinal injury, we crossed MyD88 conditional knockout mice with Col1α2cre mice (Fib-MyD88), which targets the collagen 1a promoter in cells of mesenchymal origin such as fibroblast, myofibroblasts, and mesenchymal stem cells[48](Figure 7). Deletion efficiency in Col1α2 cells was previously verified by confocal microscopy and flow cytometry [48] (Figure 7). Mice that were negative for Col1α2Cre, but positive for MyD88 recombination were used as controls. These mice were derived from at least five backcrossings with B6 mice, in order to retain a similar basal microbiota composition. After deletion of MyD88 by intraperitoneal injection of tamoxifen, 2% DSS was administered in their drinking water to mice for 5 days (Figure 8). At day eight mice were euthanized and histological analysis of harvested colonic tissue

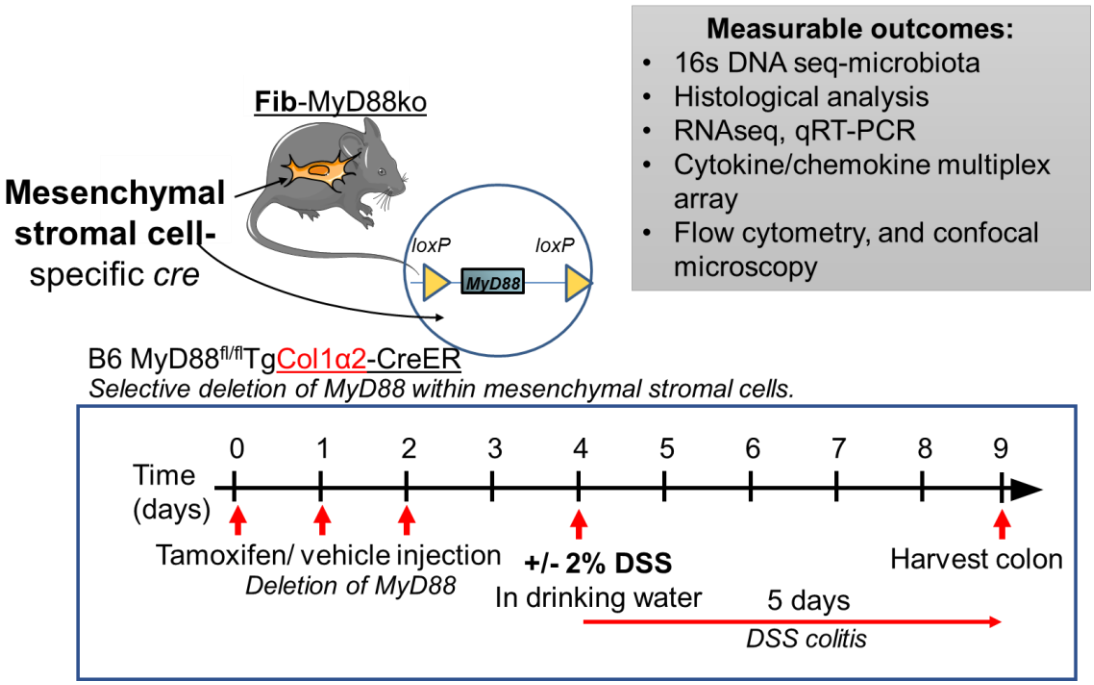

Figure 8. Fib-MyD88$^{fl/fl}$ (Col1α2Cre) mice were used for selective deletion of MyD88 within mesenchymal stromal cells. Schematic of experimental design. Control and MyD88$^{fl/fl}$Col1α2Cre mice were injected intraperitoneally with 0.05mg/100µl of tamoxifen or vehicle for 3 consecutive days (0-2). 2 days after administration of tamoxifen/vehicle, mice received 2%DSS in their drinking water or regular drinking water for 5 days (4-9). Mice were euthanized on the 10th day (9) and colonic tissue was harvested. Fecal samples were analyzed by 16s DNA sequencing for microbiota analysis. Colonic mucosal sections were stained with H&E and Trichrome for histological analysis and with antibodies against molecules of interest for confocal microscopy. Colonic mucosa scrapings were used to extract RNA for RNAseq analysis and qRT-PCR. While digested colonic tissue was used for Flow cytometry.

stained with hematoxylin and eosin showed that deletion of MyD88 in 6-8 weeks-old mice results in epithelial erosion, increased infiltration of lymphocytes, and mucosal ulceration (Figure 9B). Total inflammatory score was calculated based on the length of epithelial disruption, depth of mucosal ulceration and lymphocyte infiltration and we observed increased inflammatory score and moderately aggravated DSS induced acute colitis in mice lacking Fib-MyD88 (Figure 9C). We also observed a significant increase in collagen deposition within the colonic mucosa(Figure 9D), which is a molecule implicated in ECM remodeling and wound healing. Its deposition results in fibrosis and formation of scar tissue, contributing to an increase in fibrotic score. Fibrotic scoring was based on percentage of the colon involved and the depth of collagen deposition in the colonic crypts. Fib-MyD88 KO mice displayed a significantly aggravated fibrotic score in comparison to their littermate

controls (Figures 9E). This response was further worsened in response to DSS-induced colitis. These results suggest an important suppressive role of the MyD88 signaling pathway in mesenchymal stromal cells in the maintenance of homeostasis and in response to injury.

Mice lacking Myd88 in stromal cells have abnormalities in microbiota that mirror IBD-like dysbiosis

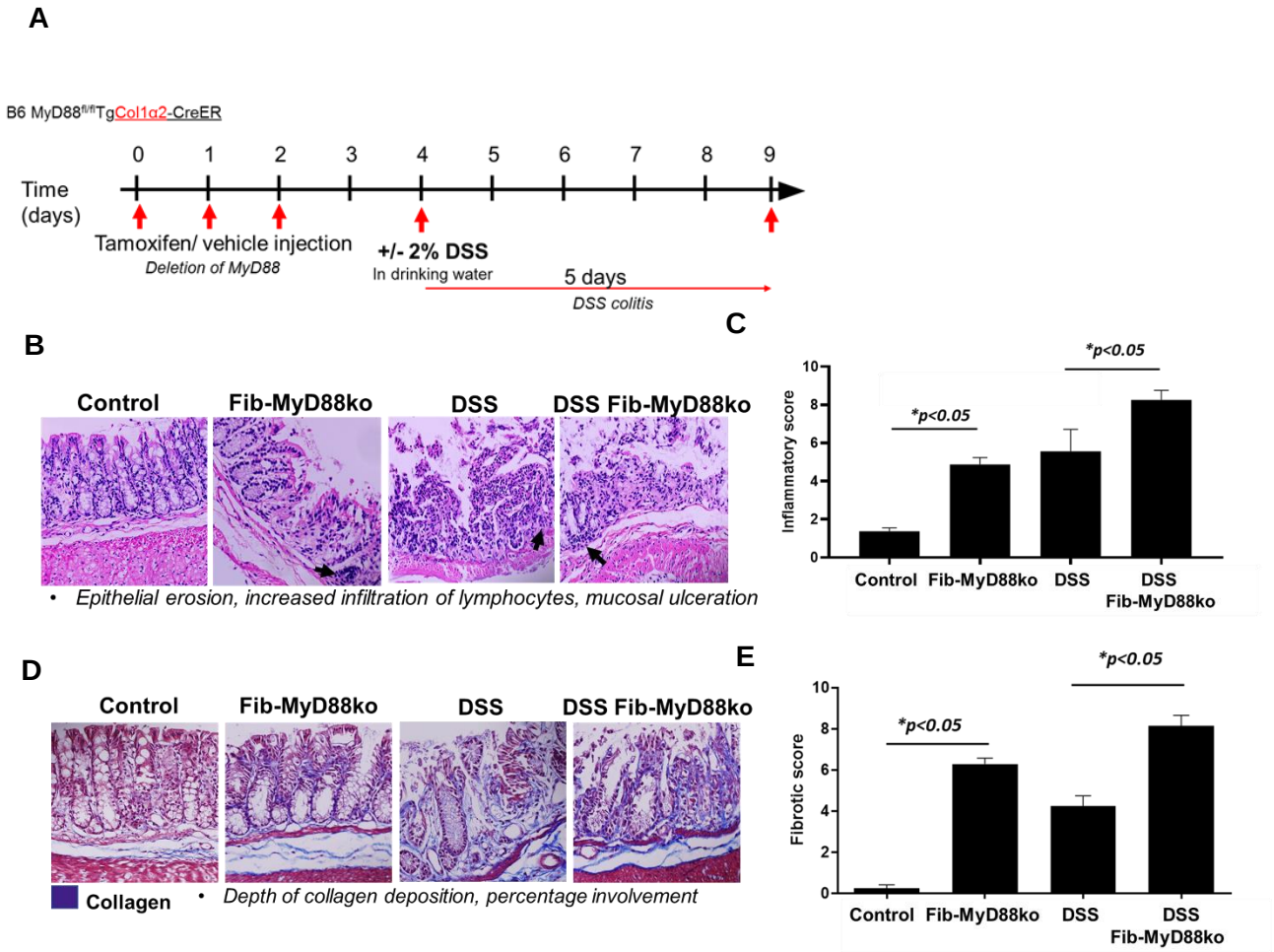

Figure 9. Deletion of MyD88 within stromal cells increases inflammatory responses and aggravates mucosal damage and fibrosis in DSS colitis. Histopathological analysis of colonic tissue in mice lacking MyD88 in MFs under homeostasis and DSS treatment. **(**A) Deletion of MyD88 within mesenchymal stromal cells in both KO models resulted in the inflammatory changes and infiltration of lymphocytes within colonic mucosa and moderately aggravated DSS induced acute colitis. (B) colon length when compared to DSS treated and untreated WT controls. Representative (C) H&E staining of colonic tissue sections show epithelial disruption, mucosal ulceration and lymphocyte infiltration in Fib-MyD88ko when compared to WT. (D) Histopathological analysis of DSS treated Fib-MyD88ko and WT Fib-MyD88ko. (E) Trichrome staining and (F) fibrotic scoring of colonic tissue sections of untreated and DSS treated WT and Fib-MyD88 ko mice.

Another compounding factor that further aggravates mucosal damage is dysbiosis of the gut microbiota. Because MyD88 is critical for microbiota signaling, we first analyzed if deletion of MyD88 from stromal cells had any effects on the fecal microbiota. This analysis was carried out by using 16s DNA sequencing. We observed that mice lacking

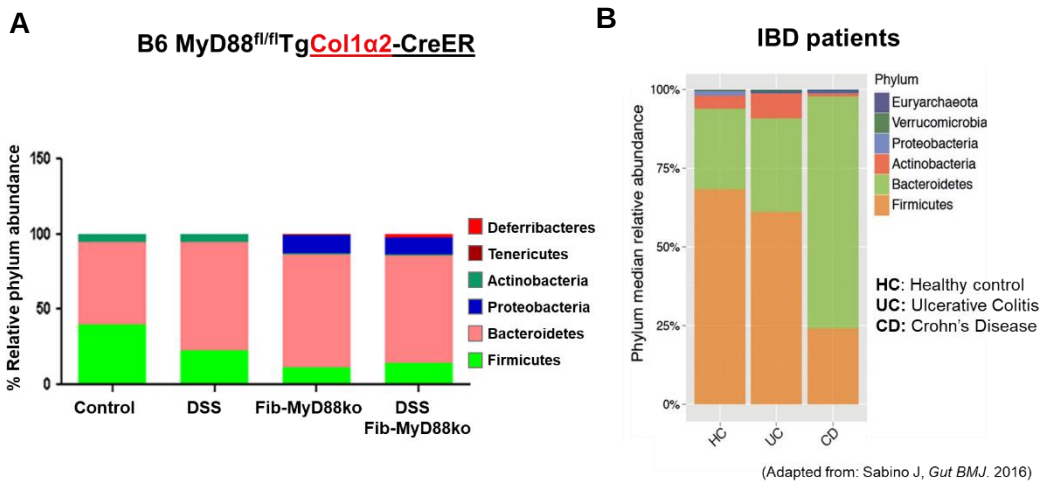

Figure 10. Mice lacking MyD88 in stromal cells have abnormalities in microbiota that mirror IBD-like dysbiosis. Microbial population are altered in mice lacking MyD88 within mesenchymal stromal cells in both homeostasis and DSS colitis that mirror changes in the microbiota of IBD patients. (A) Measurement of changes in Defferbacteres, Tenericutes, Actinobacteria, Proteobacteria, Bacteroides, and Firmicutes phyla by16s DNA sequencing of the fecal microbiota in mice lacking MyD88 within MFs, versus controls under homeostasis and in DSS induced colitis. (B)Phylum-level median relative abundances of the fecal microbiota composition in healthy controls, patients with UC, and patients with CD. Adapted from: *Sabino J, Vieira-Silva S, Machiels K, et al. Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBDGut 2016;65:1681-1689.*

MyD88 in stromal cells have abnormalities in microbiota that mirror IBD-like dysbiosis, at the phylum level (Figure 10). In particular, we observed a decrease in Firmicutes and increase in Bacteroides phyla.

Normal microbiota stimulates the increase in COX-2 and PGE_2 within the eicosanoid pathway but not 5-LO in human colonic myofibroblasts (CMFs).

COX-2/PGE_2 is critical modulator of tight junction protein expression, epithelial cell proliferation and thus maintenance of the intestinal barrier. Alterations in these signaling

processes influence the gut microbiota and modulate immune functions. CMFs are known to be the major producers of COX-2-dependent PGE_2 in the normal colonic mucosa [59]. Thus, we determined the contribution of CMFs to microbiota-induced PGE_2 synthesis. Probiotics have been clinically evaluated for use as treatment of IBD, irritable bowel syndrome, and diarrhea associated with the use of antibiotics [105-107]. Among the probiotics used, studies using *Lactobacillus rhamnosus* GG (LbGG), a bacteria known to be a part of the normal microbiota, has been demonstrated to promote anti-apoptotic and anti-inflammatory activity in mice [58, 108, 109]. More recent studies demonstrated that LbGG induced COX-2/PGE_2 production via TLR2 in neoplastic colonic epithelial cells [58]. For this reason, we chose LbGG as a representative of a "good" commensal to co-culture with normal primary human CMF isolates to explore its effect on normal stromal cells. . We observed that 24h treatment with LbGG led to a significant increase in both cPLA2 and COX-2 protein levels in CMFs (Figure 11A-B). In contrast, 5-LO enzyme production was slightly decreased (Figure 11C). Moreover, CMFs responded to stimulation by LbGG with an increase in PGE_2 production (Figure 11D). These results suggest that LbGG preferentially induced COX-2 and PGE_2 rather than 5-LO mediated leukotriene synthesis in CMFs.

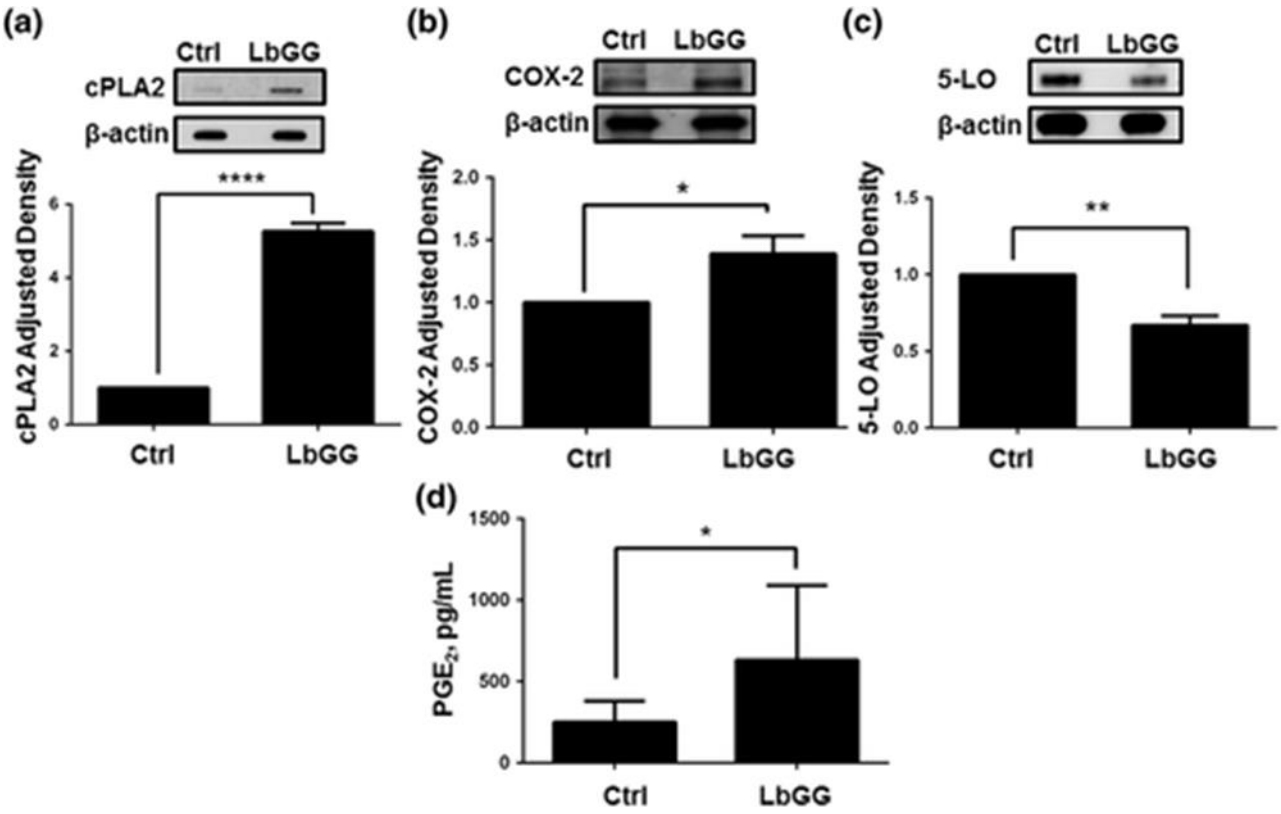

Figure 11. LbGG activated the COX-2-dependent PGE_2 pathway, but not 5-LO pathway in primary human CMFs. Primary CMFs isolated from normal colonic mucosa were exposed to LbGG for 24 hr at a ratio of 10 bacteria per one CMF. Western blotting (WB) using antibodies specific to (a) cPLA2, (b) COX-2, and (c) 5-lipoxygenase proteins demonstrated that the total cPLA2 and COX-2 levels are increased in LbGG-treated CMFs cultures compared with the untreated controls. The representative WB and summary of the adjusted density are shown. The summary of adjusted density results for each studied molecule is shown as means ± SEM. Student's t test was used to calculate the significance of the obtained results n = 3, *p < 0.05; **p < 0.01; ****p < 0.0001. (d) PGE2 concentrations determined by ELISA were increased in the condition media of CMFs after exposure to LbGG. Student's t test (Panels a, b, c) and Wilcoxon signed-ranked test (Panel d) were used to calculate the significance of the obtained results. Results from three experiments running in duplicate are shown as means ± SEM, n = 6, *p < 0.05. CMF: CD90+ myofibroblasts/fibroblast; COX-2: cyclooxygenase-2; cPLA2: cytosolic phospholipase A2; LbGG: Lactobacillus rhamnosus GG; PGE2: prostaglandin E2

Normal microbiota-induced COX-2 upregulation in CMFs is MyD88-dependent.

Because LbGG is reported to protect the murine intestinal epithelium from radiation injury through a MyD88/COX-2-dependent mechanism [58], we analyzed whether the MyD88 adaptor is required for LbGG-induced modulation of COX-2 in human CMFs. Using a siRNA approach, we demonstrated that silencing of the *myd88* gene in CMFs abrogated the LbGG induced upregulation of COX-2 protein expression (Figure 12).

These data suggest that LbGG-induced increase of COX-2 in human CMFs likely involves signaling through Toll-Like Receptors (TLRs) that requires MyD88.

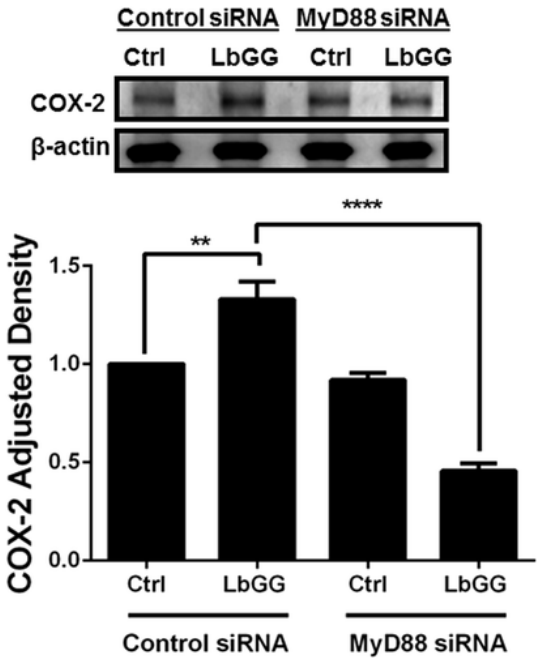

Figure 12. Silencing of myD88 gene expression in normal primary human isolates of CMFs inhibits the increase in LbGG-dependent COX-2 production. CMFs were transfected with MyD88 siRNA or control siRNA 10 days prior to exposure to LbGG. COX-2 expression was analyzed 24 hr post-LbGG exposure using Western blot (WB) analysis. A representative WB and summary of WB adjusted density analysis for COX-2 expression are shown. One-way analysis of variance was used to calculate the significance of the obtained results. Results are shown as means ± SEM, n = 3, **p < .01 and ****p < 0.0001. CMF: CD90+ myofibroblasts/fibroblast; COX-2: cyclooxygenase-2; LbGG: Lactobacillus rhamnosus GG

Normal microbiota activates the AA metabolic pathway *in vivo*

LbGG has been shown to up-regulate COX-2 and PGE_2 in murine models of intestinal injury. However, little is known about the effect of lactobacilli on this pathway under colonic homeostasis. Our results obtained with normal human CMFs strongly suggest that under homeostasis CMFs respond to LbGG with an increase in AA mobilizing enzyme $cPLA_2$ and COX-2 protein expression. Next, we determined whether LbGG can stimulate AA release and upregulate COX-2 during colonic homeostasis *in vivo*. For this purpose, $5x10^8$ CFU/dose of LbGG were administrated daily by oral gavage to C57BL/6

mice for 5 days and AA and COX-2 levels were measured in the colonic mucosa. We observed that LbGG administration resulted in an increase in colonic mucosal levels of AA (Figure 13A) and an increase in total colonic mucosal COX-2 protein expression (Figure 13B). Using COX-2 specific immunostaining followed by confocal microscopy, we confirmed results obtained by WB and observed a significant increase in COX-2 expression (in red) in the colonic mucosa of mice treated with LbGG when compared to the control group (Figure 13D). Interestingly, the LbGG-induced increase of COX-2 is mostly observed in the mucosal *lamina propria*, while only sparse and an occasional increase in COX-2 expression was seen in the colonic epithelium. CMFs are a major component of the colonic mucosal *lamina propria* [39, 96, 110]. To clarify our *in vitro* and *in vivo* observations, we determined whether CMFs are among the major cells that increase COX-2 expression in response to LbGG administration *in vivo*. Mouse colonic mucosa sections were stained with anti-α-smooth muscle actin (α-SMA) mAbs that identify the activated subset of CMFs (shown in green) and anti-COX-2 mAbs (red). We observed that a significant fraction of the COX-2-expressing cells are α-SMA^{+} (Figure 13C, orange-yellow color formation on the merged images) in the *lamina propria* of the LbGG treated group. This suggests that, during homeostasis, CMFs are important contributors to LbGG-induced COX-2 expression in the colonic mucosa.

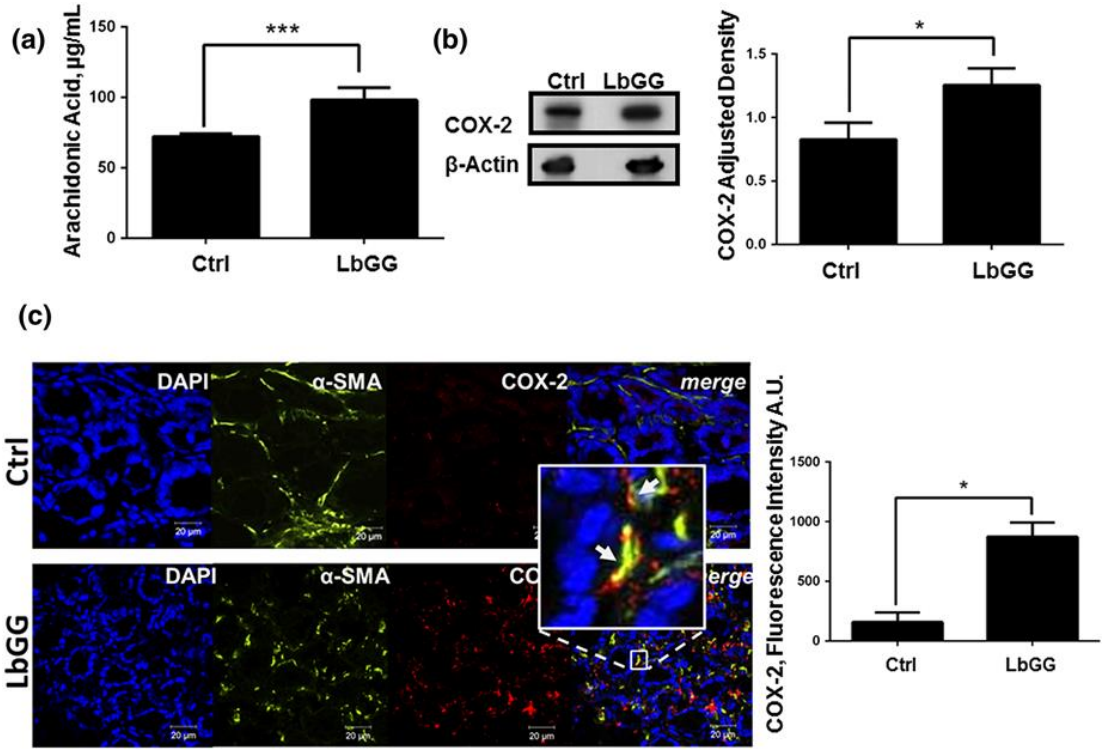

Figure 13. **LbGG activates the arachidonic acid (AA) metabolic pathway in vivo and increased COX-2 expression in lamina propria aSMA+ CMFs**. 5 × 10^8 CFU/dose of the LbGG was delivered daily by oral gavage for 5 days to C57BL/6 mice. (a) AA levels in the harvested, homogenized mucosa were determined by ELISA. Exposure to LbGG significantly enhanced the release of AA in the colonic mucosa of LbGG-treated mice. The results are shown as means ± SEM, n = 5, ***p < 0.001. (b) Western blot analysis demonstrated an increase in COX-2 expression in the colonic mucosa of LbGG-treated mice. The results are shown as means ± SEM, n = 6, *p < 0.05. (c) Representative tissue sections of the colonic mucosa from control and LbGG treated mice were immunostained and analyzed by confocal microscopy (see Section 4). Expression of COX-2 is increased within the α-SMA+ CMFs in the colonic mucosa of mice treated with LbGG. DAPI was used to stain cell nuclei (blue); activated CMFs were detected by anti-α-SMA mAb (green; clone A4); anti-COX-2 mAb (red; clone 33) was also used. A yellow-orange color on merged images indicates colocalization of α-SMA and COX-2 (indicated by arrows). Scale bar represents 20 µm. Representative cross sections are shown, n = 5 animals per group. (d) The summary of changes in the corrected total cell fluorescence intensity from in situ COX-2 protein expression in the LbGG-treated and control murine colonic mucosa. Student's t test was used to calculate the significance of the obtained results. The means ± SEM are shown, n = 5 per group **p < 0.01. CMF: CD90+ myofibroblasts/fibroblast; COX-2: cyclooxygenase-2; LbGG: Lactobacillus rhamnosus GG

MyD88 signaling in α-SMA$^+$ CMFs is required for COX-2 production in response to normal microbiota *in vivo.*

Our experiments in culture demonstrated that LbGG induced the upregulation of COX-2 in CMFs in a MyD88 dependent manner. This observation raised the question whether MyD88 is required for COX-2 expression in CMFs in response to LbGG *in vivo*. In the colonic *lamina propria*, α-SMA is expressed by myofibroblasts in the stroma. Therefore, we generated an α-SMA-specific tamoxifen-inducible conditional KO mouse selective for MyD88 (MF-MyD88 KO). The α-SMA-specific tamoxifen-inducible conditional KO mouse was used solely in this experiment. Selective deletion of MyD88 in MFs was accomplished in the same manner as Col1α2-Cre mice (Figure 8). The specificity of MyD88 deletion within the α-SMA^{+}CMFs, as well as retained MyD88 expression in other innate immune cells (such as CD11c+ dendritic cells, F4/80+ macrophages) within the *lamina propria* was confirmed by confocal microscopy analysis (Figure 7). Additionally, we did not observe any change in MyD88 expression in the epithelial compartment of the murine colonic mucosa, which was identified by location (first layer from lumen).

Using immunostaining followed by multi-color confocal microscopy in this animal model, we observed that deletion of MyD88 within the CMFs resulted in an overall reduction in COX-2 protein expression (in red) within the mucosal stroma (lamina propria) *in situ* (Figure 14A-B). This was further confirmed by western blot analysis of total colonic mucosal tissue (Figure 14C). Finally, these results taken together with our data with human cultured CMFs, suggest that under homeostasis, administration intraluminally and in vivo of LbGG may induce mobilization of AA and increase in COX-2 level in a MyD88-dependent manner in colonic myofibroblasts (stromal cells).

In Summary, our data generated under the Aim1 demonstrated that:

- Under homeostasis, mesenchymal stromal cell- intrinsic MyD88 may contribute to epithelial barrier integrity/permeability through its regulation of molecules involved in EB maintenance and repair (COX-2-mediated-PGE2).

- Deletion of MyD88 from myofibroblasts resulted in shifted microbial populations that resemble that seen in IBD patients and this may contribute to the inflammatory milieu resulting in observations above.

- The absence of MyD88 in mesenchymal stromal cells of the mouse further aggravates mucosal damage in an epithelial injury model

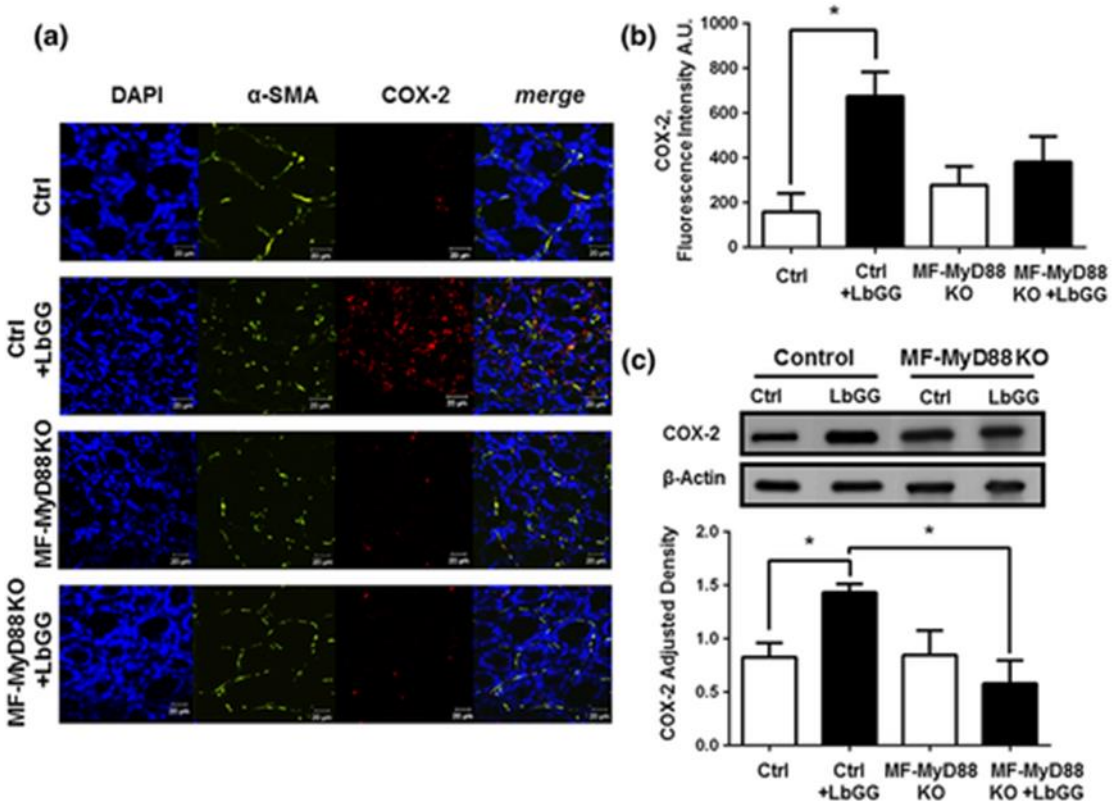

Figure 14. **MyD88 signaling in α-SMA+ CMFs is required for COX-2 production in response to LbGG**. 5 × 10^8 CFU/dose of the LbGG was delivered daily by oral gavage for 5 days to MF-MyD88 KO or control mice. Control mice negative for Acta2-Cre but positive for MyD88fl/fl recombination were derived from at least five backcrossing of MF-MyD88 KO with C57BL/6 animals. (a) Immunostaining of colonic mucosal tissue cross sections was performed, followed by confocal microscopy. DAPI was used to stain cell nuclei (blue); activated CMFs were detected by anti-α-SMA mAb (green; clone A4) and the sections were stained for COX-2 with mAb (red; clone 33). Scale bar represents 20 μm. (b) Measurement of corrected total cell fluorescence intensity from confocal microscopy images of COX-2 protein and (c) Western blotting using antibodies specific to COX-2 demonstrated that the total COX-2 levels increased in colonic tissue of LbGG-treated mice compared with the untreated controls. Deletion of MyD88 within CMFs reduced this response. One-way analysis of variance was used to calculate the significance of the obtained results. Results are shown as means ± SEM. Four–five animals per group were used in the above experiments, *p < .05. CMF: CD90+ myofibroblasts/fibroblast; COX-2: cyclooxygenase-2; LbGG: Lactobacillus rhamnosus GG

The resulting changes in microbiota composition, along with alterations in COX-2/PGE_2 signaling upon deletion of MyD88 in MFs, suggests that MFs may play a critical role in the regulation of signals initiated by the microbiota, and the consequence of the changes may have a downstream effect on the abundance of microbial populations. While we are unable to determine how fibrosis is increased when MyD88 is inactivated in the cells that are the major producers of collagen, it may be possible that these global changes are attributed to non-cell autonomous event. Alternatively, there may be changes in the expression of other molecules in newly recruited macrophages or MFs resulting from deletion of MyD88 in MFs. For example, our lab recently found that MFs isolated from CD tissue show a higher level of matrix metalloproteinase (MMP)-7 , 9, and MMP-10 (enzymes responsible for the degradation of ECM) mRNA when stimulated with lipopolysaccharide (cell wall component of gram negative bacteria) (Aguirre J, under revision). However, this response occurred with a functionally active microbiota mediated-MyD88 signal. While disruption of MyD88 may hinder the increase of ECM-degrading MMPs, this does not fully explain the significant increase in collagen resulting from the deletion of MyD88 in MFs. On the other hand, macrophage have also been shown to produce collagen and secrete ECM proteins[111]. Moreover, while macrophages have also been correlated with increased MMP expression in IBD, Crohn's disease endoscopic index of severity (CDEIS) value have been shown to positively correlate with macrophage TIMP-1[112] (inhibitors of MMPs). Therefore, these compounding factors may lead to the dysregulated response to increased collagen deposition in our model. While the source of collagen in response to MF-MyD88 deletion remains unknown, and while we are uncertain if other innate immune cells may contribute to this increased inflammatory response, we are certain that there is cross-talk between MFs and macrophage (see Chapter 3). Therefore, having a better understanding of how these cells influence one-another may give us more insight on the mechanisms leading to these processes.

CHAPTER 3

MF INTRINSIC MYD88 SIGNALING SUPPRESSES INFLAMMATION IN THE COLONIC MUCOSA BY INHIBITING THE INFLUX OF INFLAMMATORY MACROPHAGES

Preliminary data, aims, and hypothesis

Signaling through MyD88 in innate immune cells is critically involved in eliciting and regulating immune responses to the microbiota in the colonic mucosa under homeostasis and in inflammatory bowel disease (IBD). MyD88 signaling in intestinal macrophages is implicated in the regulation of inflammatory responses in the colonic mucosa and involve mesenchymal stromal cells. MyD88-dependent signaling in mesenchymal stromal cells (fibroblasts, myofibroblasts and their progenitors) are reported to be involved in epithelial barrier restoration and in tolerance by controlling TLR- and type 1-inflammatory responses. However, the role of MyD88-dependent signaling by mesenchymal stromal cells in the regulation of inflammatory responses by macrophages in the colonic mucosa is poorly understood. Because we observe that colonic mesenchymal stromal cells respond to MyD88 activation with production of molecules involved in the regulation of macrophages (PGE2, PD-L1, etc.)[48, 113] we hypothesize that stromal cell-intrinsic MyD88 signaling contributes to the suppression of inflammatory responses by innate immune cells in homeostasis and IBD-like colitis.

Results

MF intrinsic MyD88 signaling suppresses expression of markers of macrophages and inflammatory cytokines implicated in the pathogenesis of IBD

To identify the changes in MF intrinsic MyD88 gene expression, we performed RNA sequencing of the colonic mucosa from control and Fib-MyD88 knockout mice treated with or without DSS. A representative heatmap of the RNAseq data demonstrated that Fib-MyD88 knockout (KO) mice had a significantly altered gene expression profile in comparison to the control mice (Figure 15A). Comparison of untreated control and Fib-MyD88KO mice with the DSS treated control and Fib-MyD88KO samples showed that 307 pathways within the colonic mucosae require MF-MyD88 signaling under homeostasis and in DSS colitis (Figure 15B). An Ingenuity Pathway Analysis (IPA) of changes in these MyD88-dependent pathways revealed differences related to inflammation and immune responses, indicating the failure of these mice to acquire an innate identity under homeostasis (Figure 15C).

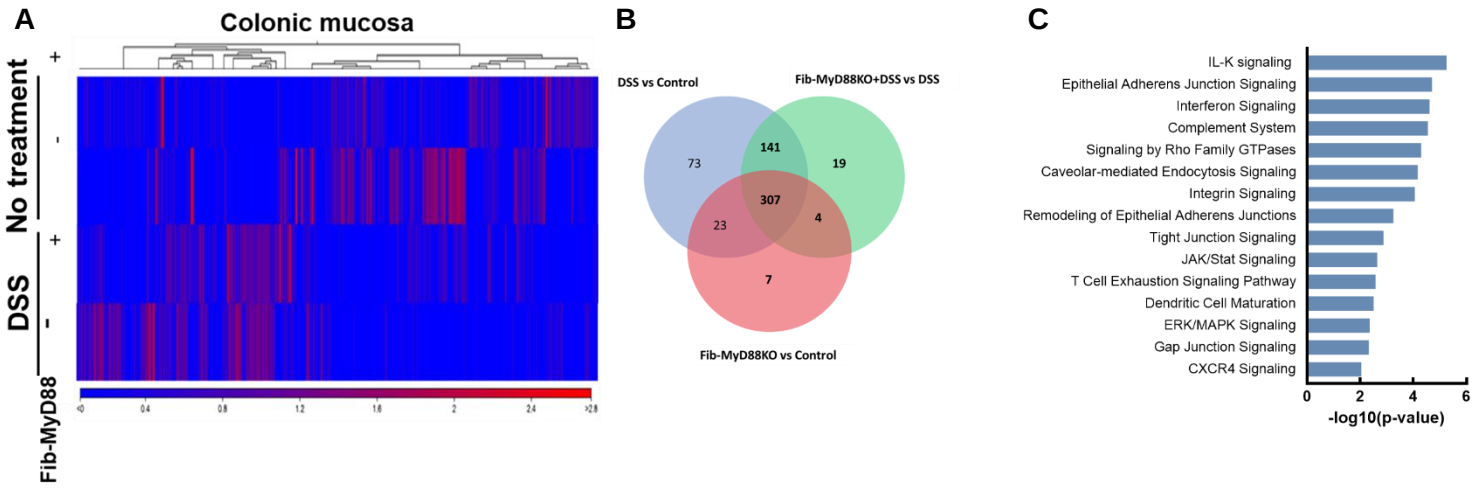

Figure 15. **Deletion of MyD88 in stromal cells results in dysregulation of pathways involved in the regulation of the EB.** Changes in gene expression profile in the colonic mucosa of mice lacking MyD88 in MFs. A) Representative heatmap of differentially expressed genes in DSS treated or non-treated control and Fib-MyD88 KO mouse colonic mucosa. Ingenuity pathway analysis used to develop a B) Venn diagram showing the number of overlapping differentially regulated pathways between untreated and DSS treated control and Fib-MyD88 KO.C) Pathways altered by MyD88 indicated as bold in (B).

To further analyze the differences between DSS treated or untreated control and Fib-MyD88KO mice, we next analyzed the expression of genes associated with inflammatory

responses. We found significant changes in many genes, particularly those encoding innate immune mediators, and genes associated with the macrophage repertoire. We observed in increase in IL-1β, IL-6 and TNF-α genes of inflammatory cytokines produced by macrophage populations in both DSS treated and untreated Fib MyD88KO mice (Figure 16). Among the altered genes were elevated levels of CD11b, F480, and CD209f, macrophage surface markers with important functions in differentiating macrophage phenotypes, in the colonic mucosa of mice lacking MyD88 in MFs compared to controls, and this response was further aggravated in mice treated with DSS (Figure not shown). We then validated the increase in the pan marker of gut macrophage F4/80 using qRT-PCR. We observed an increase in *f480* mRNA in both untreated and DSS treated mice lacking MyD88 in MFs (Figure not shown). Thus, deletion of MyD88 in stromal cells appears to result in an increase in lamina propria macrophages.

A

Alias	Fold change	P-value
IL-6	2.770961	0.387305
IL-1β	1.325085	0.604443
TNF-α	3.845343	9.22E-05

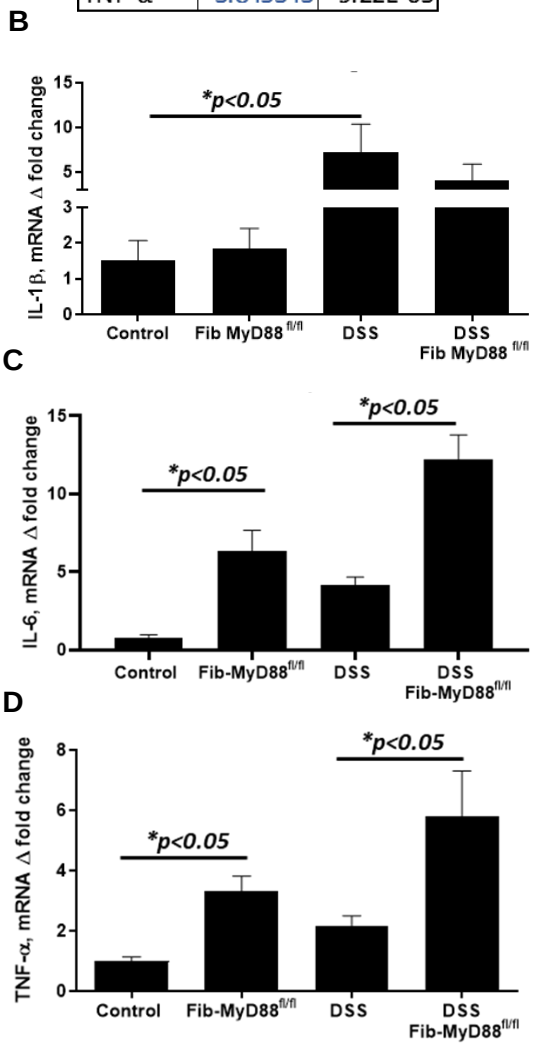

Figure 16. Deletion of MyD88 in stromal cells results in increased expression of inflammatory cytokines in murine colonic mucosa (A) Fold expression of inflammatory cytokine known to be produced by macrophages and confirmatory RT-PCR measuring mRNA fold change of (b) Il-1β, (C) IL-6, and (C) TNF-α. Data represent mean ± SEM. ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001,

Since there was significant differential expression of pro-inflammatory genes in the colonic mucosa of Fib-MyD88 KO mice, we also measured the infiltration of inflammatory cells by flow cytometry. We observed a statistically significant increase in cell populations bearing $CD11b^{+}/F480^{+}$ markers, but lacking CD11c, CD11c is

predominantly a dendritic cell marker). Thus the infiltrating cells are of the macrophage phenotype (Figure not shown). Therefore, these data suggested that Fib-MyD88KO mice display altered immune regulation toward a more pro-inflammatory microenvironment with an enhanced number of inflammatory macrophage. Taken together, these results indicate that MF-intrinsic MyD88 signaling is critical to the regulation of inflammatory responses in the colon by suppressing the influx of inflammatory macrophages under homeostasis and during initiation of the inflammation.

My88 signaling in MFs suppress migration of inflammatory macrophages to the colonic mucosa

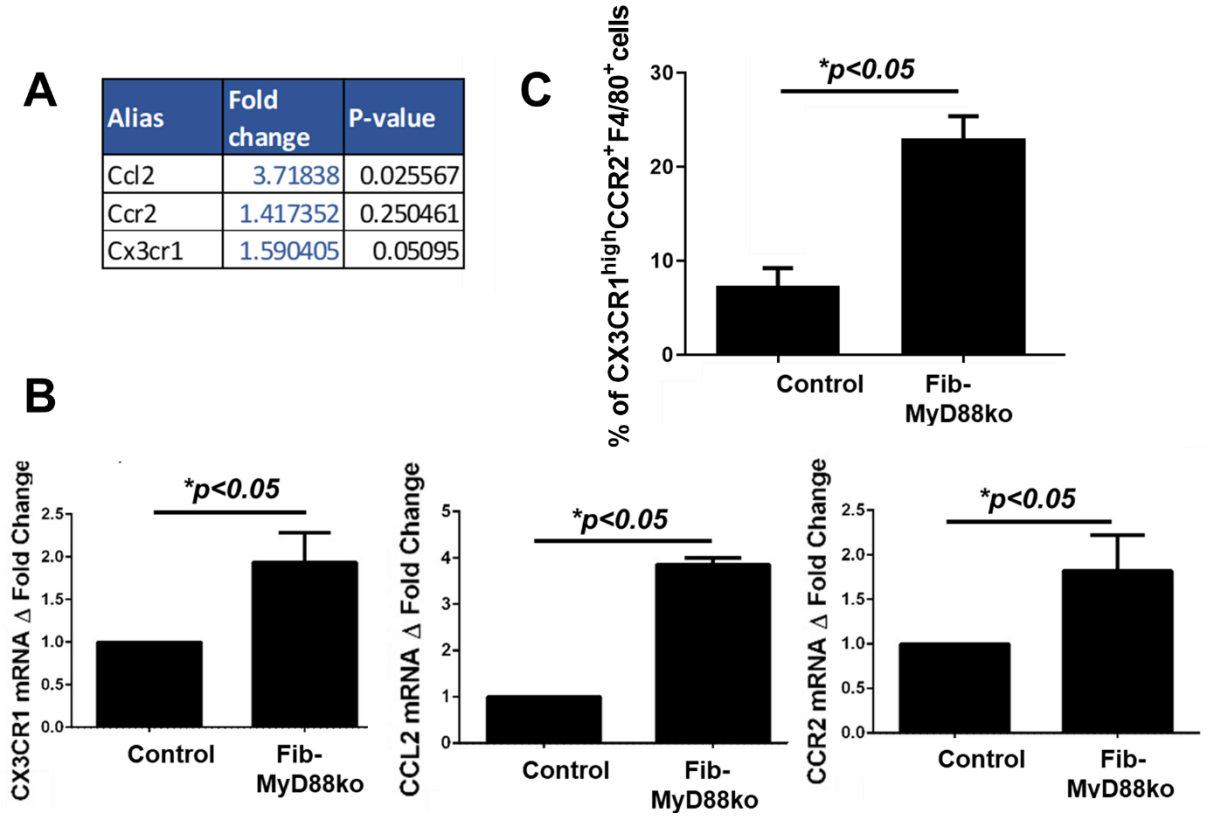

Figure 17. Deletion of MyD88 in stromal cells results in increased chemokine expression involved in the influx of monocytes. CX3CR1^{high}CCR2^{+} cells producing TNF-α were predominant within the macrophage populations in mice lacking MyD88 in stromal cells.(A) RNAseq fold expression and (B) qPCR of chemokines and their respective receptors that are associated with macrophage homing to the gut. (C) Human colonic macrpophages were identified and characterized for the expression of F480, CX3CR1, and CCR2. Flow cytometry demonstrated an increase of cells bearing CX3CR1^{high}CCR2^{+}F480^{+} markers. Data represent mean ± SEM. ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001.

Murine intestinal macrophages in the *lamina propria* preferentially express CX3CR1 chemokine receptor [114]. CX_3CR1 is a specific marker for *lamina propria* macrophages and a critical component in maintaining homeostasis in *lamina propria* [114].While interactions of CX_3CR1 are believed to be important for maintaining an anti-inflammatory environment in the *lamina propria*, the CCR2/CCL2 and/or other chemokine axes are believed to regulate the accumulation of proinflammatory macrophages in the inflamed mucosa.[32, 115, 116] In IBD, macrophages phenotypes and distribution are distinct from normal tissue resident macrophages in homeostasis. While the exact mechanism of action is not well known, the CX_3CL1/CX_3CR1 chemokine axis is also strongly associated with IBD. Specifically, the increase of CX_3CL1 transcription is associated with the inflamed lesions of the colonic mucosa in patients with CD [117]. Peripheral blood mononuclear cells migrate to tissue sites where there is inflammation, then differentiate into pro-inflammatory macrophages as opposed to switching from ant-inflammatory to proinflammatory macrophage phenotype. However, there are number of limitations in proving chemotaxis *in* vivo. Having observed an increase in $F4/80^+$ macrophages in the inflamed colonic mucosa of mice when MyD88 was deleted from MFs, we performed experiments to determine if CX3CR1 was increased and whether CCR2 influenced the accumulation of macrophages in the colonic mucosa.

RNAseq analysis of the murine intestinal *lamina propria* demonstrated an increase in the expression of CX3CR1, CCL2, and its putative receptor CCR2 (Figure 17A). The total cell number of $CX3CR1^{high}CCR2^+F480^+$ macrophages was increased (Figure17C) in the colonic mucosa of mice lacking MyD88 in MFs in both homeostasis and in DSS colitis model. This mimics several inflammatory processes observed in IBD. We also observe that these cells were $CD11b^+$, a marker shared between monocytes and macrophages.

Furthermore, tissue-infiltrating intestinal macrophages have been shown to produce pro-inflammatory cytokines such as TNF, IL-6, IL-8, IL-23, IL-1β, and IFNγ as well as the chemokine CCL2 [118-120]. Among the accumulated macrophages in the colonic mucosa following deletion of MyD88 in MFs, we were curious to determine if they contributed to the

pro-inflammatory environment through the production of these cytokines. We observed an increase in macrophage cell populations bearing $Cd11b^{+}F480^{+}$ that are known produce TNF-α and IL-6 , but not $CD11c^{-}$ dendritic cells(Figure 18A and B).

This suggests that MFs interaction with the microbiota toll like receptors and/or through the IL-1 receptor is critical to their role as sentinels of intestinal homeostasis through the suppression of the influx of the monocyte derived $CCR2^{+}$ macrophages.

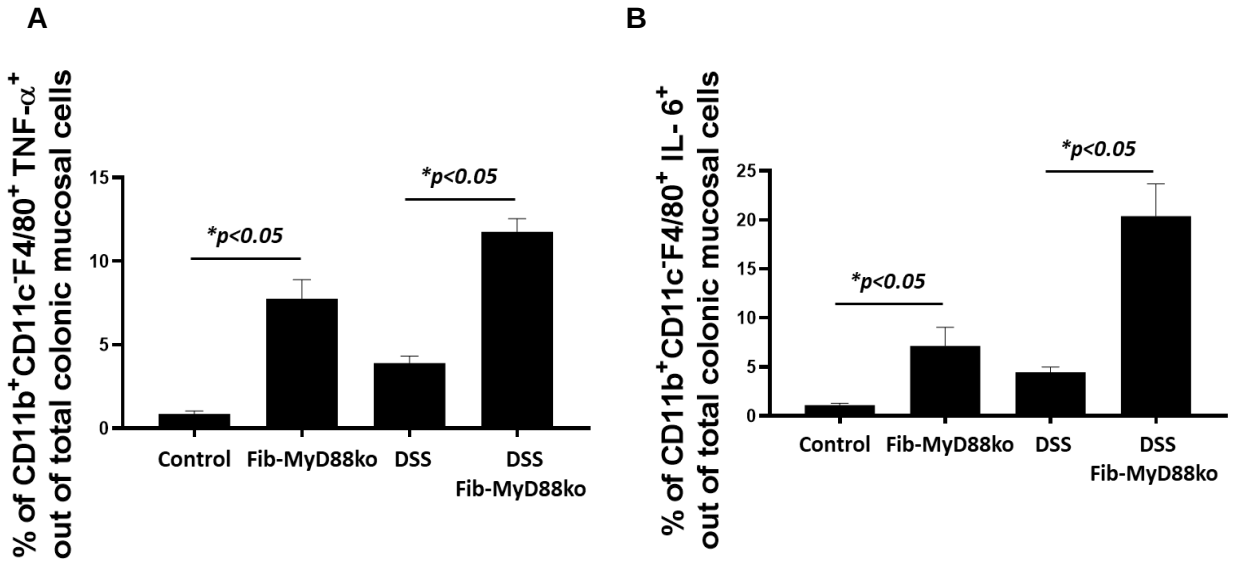

Figure 18 . Loss of MyD88 in stromal cells results in increases inTNF-α and IL-6 producing macrophages. Flow cytometry demonstrates an increase of cells bearing $CD11b^{+}F480^{+}CD11c^{-}$ markers expressing (A)TNF and (B) IL-6 in mice lacking MyD88 in mesenchymal stromal cells. Data represent mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Migration of inflammatory macrophages are critical to inflammatory responses observed in mice lacking mf intrinsic MyD88

Although the underlying cause of IBD is not completely understood, it is known that TNF-α is an early potent pro-inflammatory cytokine in the inflammatory processes underlying IBD.[121] Clinical studies demonstrating a significant therapeutic response from the neutralization of TNF with antibodies implicated a significant role for TNF-α in the pathogenesis of IBD.[122] Because macrophages are major contributors of TNF-α [123], we next determined if macrophages were a major source of TNF-α in the colonic mucosa of our model. Depletion of macrophages with the use of parenteral clondronate liposomes (FormuMax) [124](Figure 19A and B) results in a dramatic decrease of the TNF-α producing cells (Figure 19C). Subsequently, we confirmed that these inflammatory macrophage populations resulting from the deletion of MyD88 from MFs were critical to the increase of total TNF-α in the colonic mucosa (Figure 19C). Whether the inflammatory changes observed in chapter 1 were attributed by these macrophages will require further histological analysis of the colonic mucosa of mice lacking MyD88 in MFs. These observations suggests that the accumulated inflammatory macrophages in the colonic mucosa is likely to be a critical process negatively regulated by the MF intrinsic MyD88. Taken together, these data suggests that MFs may play a critical role in the suppression of newly migrated, TNF-a producing inflammatory macrophages to the colonic mucosa through the modulation of stromal cell MyD88 signaling. The contribution of MF-intrinsic MyD88 signaling to the regulation of TNF-α producing macrophages, may have major implications in responsiveness to anti-TNF therapy.

Deletion of MyD88 within MFs leads to changes in transcription that demonstrate a pro-inflammatory signature, as well as those involved in the maintenance of the epithelial barrier. While we are unable to pinpoint the exact effects of MyD88 on global transcriptional changes, and in turn specific pathways effected, we were able to definitively implicate the critical pathways as those that are associated with alterations in MF-MyD88 signaling. Ultimately, changes in macrophage-associated pathways appeared to be a critical component for gut inflammation. We were able to confirm the role of macrophage in perpetuating/initiating inflammation through the production of pro-inflammatory cytokines. Further, it appears that is *de novo* migrating macrophages that are critical to the overall increase in colonic TNF-α. This suggests that MF-macrophage interplay is critical to the

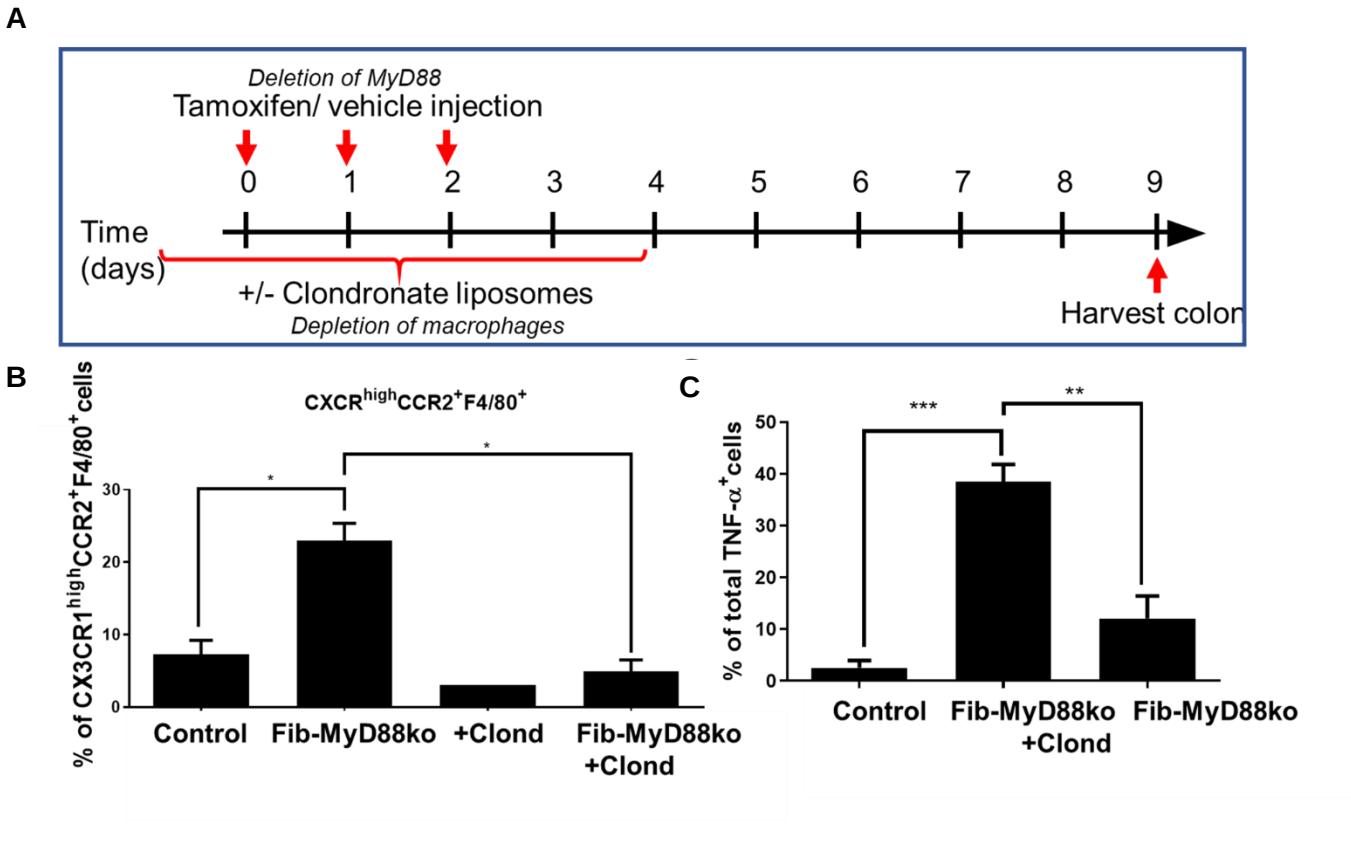

Figure 19. **Depletion of macrophages with anionic liposomal clodronate blocks macrophage-induced TNF-α increase in mice lacking MyD88 in stromal cells.** (A) Clodronate liposomes (7 mg/mL) were given intraperitoneally to control and Fib-MyD88KO mice (200 µl/20 g mouse) at 4 days, (100 µl/20 g mouse) at 2 days, (100 µl/20 g mouse) at 8 hours before, and finally 100 µl/20 g mouse) at 2 days after 3 consecutive days of intraperitoneal tamoxifen injections. Data are means±SEM for 5 mice per group. **P<0.001, ***p<0.0001 compared with controls. (B) Flow cytometry of cells bearing CX3CR1^{high} CCR2^{+}F480^{+} markers or (C)TNF-α.

maintenance of colonic homeostasis. Although we cannot exclude that other innate immune cells may contribute to these changes, these data and supporting literature provide a platform to further elucidate the critical role of MFs interaction with the microbiota in maintaining colonic homeostasis.

CHAPTER 4

OVERALL DISCUSSION AND CONCLUSION

My dissertation research focuses on the contribution of MF-intrinsic MyD88 signaling to the maintenance of colonic homeostasis and how this signaling is involved in the initiation of the inflammatory responses within the colonic mucosa.

Our data revealed a novel role of MF MyD88 in shaping the composition of the murine intestinal microbiota. Upon deletion of MyD88 from MFs, there is a shift in the microbiota composition that resembles IBD-like dysbiosis, with an increase in Bacteroides and decrease in Firmicutes populations. At the phyla level, Firmicutes are critical to the composition of the intestinal microbiota in healthy Individuals [125]. In IBD, dysbiosis consists of decreased proportions of Firmicutes, increase of Bacteroidetes, and increased proportions opportunistic enterobacteria [126]. Individuals with IBD have increased intestinal permeability, dysregulated immune responses and fibrosis, all of which are believed to contribute to and possibly be causative factor in the change in the proportions of the microbiota [127]. Interestingly, H&E or trichrome stained colonic sections scored for inflammation or fibrosis, respectively, were increased in mice lacking MyD88 in MFs. Increased collagen deposition is a hallmark of microscopic colitis (MC)[128]. Although this has not been studied well, evidence points to the fact that, much like IBD, MC pathology involves changes in the microbiota[129]. For instance, one study has demonstrated that a patient with UC began to develop MC following a fecal microbiota transplantation[130]. In general, there have been few studies that shed light on the potential role of the microbiota in MC. Therefore, the implications for these finding are that the interaction microbiota with the MFs via MyD88 may play a key role in not only maintaining homeostasis, but in controlling inflammatory responses that elicit dysbiosis which in turn further perpetuate inflammation in either IBD, including in MC.

Moreover, deletion of MF-MyD88 disrupted the potential interaction of the microbiota in promoting homeostasis and epithelial barrier restoration through the production of PGE_2. We demonstrate that normal human CMF isolates uniformly responded to LbGG stimulation with an increase in cPLA2, COX-2, and PGE_2 upregulation. Our in vivo study confirmed our observation in culture showing that oral gavage of healthy adult mice with LbGG for 5 days results in increased levels of AA and COX-2 protein expression in the colonic mucosa. Thus, our studies demonstrate that LbGG-mediated induction of these molecules also occurs in vivo under homeostasis. Previously, the effect of lactobacilli on upregulation of COX-2 and PGE_2 has been demonstrated in animal models of injury. For example, the use of *Lb*. *fermentum* ZYL0401 in a lipopolysaccharide-induced hepatic injury model resulted in the increased ileal expression of COX-2 and production of PGE_2 [131]. LbGG-induced COX-2-expression protected the intestinal epithelium in mice against radiation injury.[58] Interestingly, these authors did not observe an increase in COX-2 expressing cells but rather demonstrated repositioning of *lamina propria* COX-2 expressing mesenchymal stem-like cells closer to the epithelium. Mesenchymal stem cells are progenitors of CMFs but do not express α-SMA. Although our study does not exclude modulation of LbGG-induced COX-2 in mesenchymal stem cells during injury, we have shown that under homeostasis, a significant part of the increase in COX-2 expression is associated with differentiated α-SMA^+ CMFs. We previously observed that lack of Toll-like receptor/MyD88-dependent signaling in CMF abrogates inflammatory responses in vivo.[48] In the present study, we demonstrate that deletion of MyD88 in human CMFs in cultures and murine α-SMA^+ CMFs in vivo decreases LbGG-induced mucosal COX-2 levels. Although it remains to be evaluated whether subepithelial CMFs come in direct contact with the LbGG in the non-injured epithelium (homeostasis), it is likely that LbGG secreted compounds and/or bacteria cell wall components (e.g., LPS) do make contact with CMFs. In fact, Ciorba et al[58] showed that oral administration of LbGG condition media is sufficient to induce the COX-2-mediated

intestinal radioprotective effect in vivo. Further, we have shown that oral gavage of LbGG in normal mice increases COX-2 expression within CMFs in the *lamina propria*.

In this work, we highlighted the ability of the normal microbiota to induce COX-2-dependent PGE_2 production in the entire colonic mucosa and in subepithelial (myo) fibroblasts through a MyD88-dependent mechanism. Taken together with the previously described functions of PGE_2 in the colonic mucosa, our data suggest that probiotic treatment with LbGG could participate in the maintenance of epithelial barrier integrity and mucosal tolerance through increased release of COX-2-mediated PGE_2 by mucosal (myo) fibroblasts. However, the fact that some colon carcinoma epithelial cells express COX-2 and can respond to LbGG with tumorigenic PGE_2 secretion suggests that probiotics should be used with caution in patients with known colorectal cancer.

For my third chapter, we focus on the influence of MF-MyD88 signaling on the innate immune system. Our in-vitro data demonstrated that the pathways downstream of innate sensing in MFs via MyD88 involves the regulation of genes for inflammatory cells and molecules in the colonic mucosa. Among them are cells bearing F480 and CD11b markers that identify macrophages, as well as increased IL-6 and TNF-α cytokines produced by macrophages. The deletion of MyD88 in MFs led to the increased infiltration to the colonic mucosa of $CD11b^{+}F480^{+}CD11c^{-}$ inflammatory macrophages expressing TNF-α and IL-6. In active IBD, macrophages in the colon are reported to be the major source of TNFα [132, 133]. Upregulation of M1(high IL-12,low IL-10) inflammatory, macrophages are implicated in IBD and can induce tissue damage [134]([135]. In addition to IL-6 and TNF- α, other effector molecules such as chemokines and their putative receptors were regulated by MyD88 signaling in MFs. Macrophage populations bearing CX3CR1 and $CCR2^{hi}$ markers were observed to be increased in the colonic mucosa of mice lacking Myd88 in MFs. This suggest that the macrophages present in the colonic mucosa during inflammation are peripherally derived and not resident macrophages. We also demonstrated that recruited inflammatory

macrophage were critical to the cascading inflammatory response that was observed in mice lacking MyD88 in MFs. We have demonstrated that microbiota-stimulated MFs function as gatekeepers to suppress a hyper immune response brought about by an influx of proinflammatory macrophages. We also demonstrated MF-MyD88 signaling to be important in the regulation of the microbiota composition. Thus, our data suggest that MyD88 signaling within mesenchymal stromal cells contributes dysbiosis and to the maintenance of colonic mucosal homeostasis through suppression of the influx of TNF-α producing inflammatory macrophages, and alteration of this signaling is likely to contribute to IBD.

Finally, herein presented data together with our previous reports strongly suggest that MyD88 signaling within mesenchymal stromal cells contributes to colonic mucosal homeostasis and epithelial barrier maintenance through the regulation of several pathways involved in epithelial tight junction function and barrier integrity. We have shown also that stromal cell MyD88 signaling is critically involved in the control the pathways involved in the inflammatory innate and adaptive immune responses. These MyD88 restricted MF regulatory functions include production of the regulatory molecules such as PD-L1 and PGE_2 and the suppression of the influx of TNF-α and IL-6 producing inflammatory macrophages.

APPENDIX A

METHODS

Experimental Procedures

Antibodies

Rabbit polyclonal anti-human COX-2, rabbit polyclonal anti-phospho-5-lypoxygenase (5-LO) and $cPLA_2$ rabbit polyclonal antibodies (Abs), were purchased from Cayman Chemical (CA, USA), R&D (MN, USA), and Cell Signaling (MA, USA), respectively. Rabbit monoclonal anti-mouse COX-2 monoclonal Abs, mAbs (clone SP21) was purchased from Thermo Fisher Scientific (MA, USA). Unconjugated mouse anti-human COX-2 mAbs (clone 33) was purchased from BD (CA, USA) and labeled using Zenon Mouse IgG labeling kit was purchased from Invitrogen (CA, USA). Rabbit monoclonal anti-β-actin mAbs (clone RM112) was purchased from Millipore (MS, USA). Precision plus Dual Color standard, Western C standard, and StrepTactin-HRP Conjugate were purchased from BioRad, (CA, USA.). Fluorochrome-conjugated murine anti–α-smooth muscle actin (α-SMA; clone 1A4) monoclonal mAb was purchased from Sigma-Aldrich (St. Louis, MO).

Bacterial strain and culture conditions

Lactobacillus rhamnosus GG (LbGG) was isolated from probiotic Culturelle®, which was purchased from iHealth Inc. (Cromwell, CT, USA). Additionally, the following collection of strains were used in this study: *Lactobacillus casei* ATCC 334, *Lactobacillus paracasei* 20006, *Lactobacillus plantarum* LR3, *Lactobacillus acidophilus* 42, and *Lactobacillus brevis* 1 (LMBA laboratory, Bordeaux). *Lactobacillus* strains were grown in De Man, Rogosa and Sharpe (MRS) medium (BD, CA, USA) at 37°C under aerobic conditions.

Cells

Human colonic epithelial cell lines Caco-2, HT-29, HCT-116, LS-174T, and SW-480 were purchased from ATCC (Manassas, VA , USA) grown in MEM culture medium (Gibco, CA, USA) supplemented with 10% fetal calf serum (Sigma), 100 µg/ml streptomycin, 100U/ml penicillin, 2 mM L-glutamine and 1 mM non-essential amino acids. For CMF isolation, full

thickness fresh human colonic mucosa samples were obtained from discarded surgical resections under UTMB approved IRB protocol #99-061. CMFs were isolated from the normal margin of mucosal colonic tissue of patients undergoing colectomy for colon cancer as described previously and routinely used in our laboratory [96]; [136]. The purity of isolated $CD90^+$ CMFs (98-99%) was confirmed by flow cytometry, as previously described [96]. Studies were performed with primary CMF isolates at passages 4-10 and cultured as described previously [96].

Silencing of MyD88 gene in CMFs

Primary human CMFs lacking MyD88 expression were generated in our lab using Stealth™ siRNA probes (Invitrogen, CA). Negative siRNA controls with appropriate GC content were included in each experiment. An optimal concentration of each siRNA (0.3 nM) was used for each transfection. Transfection of primary cells was performed using Nucleofector™ technology (Amaxa Biosystems, MD, USA) according to the manufacturer's instructions.

Bacteria: cell co-cultures.

Human epithelial cell lines or primary human CMF isolates were seeded in 6 well plates and monitored until 95% confluency, then LbGG was added at 1:10 cell to bacteria ratio. In some experiments epithelial cell lines were grown on permeable membranes in transwells in order to form polarized cell monolayers, prior exposure to LbGG. In these experiments, 12 h cultures of LbGG growing in MRS broth were harvested and washed once with PBS, pH 6.8 by centrifugation at 503 x *g*, 4°C for 10 min. After washing the pellet, bacteria were resuspended in MEM medium and added to cell cultures as described above. For the transwell cell culture system, LbGG were added to the apical compartment of the epithelial cell monolayer.

Western Blot analysis

Western blot (WB) analysis were performed as previously described [96]. Briefly, human cells in culture were washed with ice-cold PBS and lysed in Laemmli sample buffer. While murine tissue samples were homogenized in 5 µL lysis buffer (Cell Signaling Technology) per mg tissue and homogenized. 10 µg of protein per sample were used for WB analysis. Expression differences are shown as adjusted density following normalization to β-actin. The

Image Lab software version 5.2.1 was used to calculate adjusted density. Adjusted density values for samples were calculated by dividing the relative density of each sample lane by the relative density of the untreated control for the same lane.

Arachidonic acid and PGE_2 determination

Arachidonic acid (AA) production was determined in human cell extracts by gas chromatography as previously described by a method adapted from [137]. Briefly, 10 µL of 10 mg/mL heptadecanoic acid (C17) were added to 10 million cells (pellet) as an internal standard. Lipids were extracted according to the method by [138] by homogenization of the pellet with 2:1 chloroform-methanol mixture (v/v) to a final dilution 20-fold the volume of the tissue sample. The extract was centrifuged and the supernatant was vacuum-dried before solubilization with methanol/benzene/acetyl chloride (4/1/0.5 v/v/v). After 1 h incubation at 100 °C, 2.5 mL of 6% potassium carbonate solution (w/v) was added to the mixture and centrifuged for 10 min at 3500 rpm. 1 µL of the upper phase was injected on Innowax column (L = 30 m, internal diameter = 0.25 mm, stationary phase thickness = 0.25 µm, S&W Scientific, USA) with a 2 mL/min helium flow. Chromatographic analysis was performed on a gas chromatography-mass spectrometry system QP2010 series (Shimadzu, Japan). Injection was assessed in split mode (division 1:20) at 250 °C and the column temperature fixed at 180 °C. AA was identified by comparison of relative retention times and was quantified after establishment of an arachidonic acid standard curve and comparison with the internal standard (Sigma, MO, USA). AA concentrations from murine tissue samples were also measured using the AA ELISA Kit (TSZ ELISA, USA). PGE_2 concentrations were determined using the Prostaglandin E2 Parameter Assay ELISA Kit (R&D systems) according to the manufacturer's protocol.

Confocal microscopy.

Frozen murine colon tissue sections were fixed in 1% paraformaldehyde for 20 minutes at room temperature, blocked with normal rabbit serum (1:10 in PBS) and murine serum (1:10 in PBS) for 15 min at room temperature. Sections were incubated overnight at 4°C with anti-murine COX-2 mAbs (clone33) conjugated with AF®647 (1 µg/mL). In some experiments, sections were then stained with AF® 488 conjugated anti-α-SMA mAbs (clone IA4), anti-murine CD11c mAbs (clone N418), and/or anti-murine F4/80 mAbs (clone BM8) for 2 hrs at room temperature. Each staining step was followed by six washes with PBS with Ca^{++}/Mg^{++}. The sections were then mounted in SlowFade® Gold antifade reagent with DAPI (Invitrogen,

CA, USA). Confocal microscopy was performed with a Zeiss LSM510 META laser scanning confocal microscope (Carl Zeiss, Thornwood, NY).

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston. C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). In the colonic mucosa, α-SMA is specifically expressed by MFs and Col1α2 is expressed by fibroblasts, MFs, and their progenitors. Tamoxifen-inducible MyD88 floxed mice specific to α-SMA^{+} or cells Col1α2^{+} were generated in our lab (Fib-MyD88 KO and MF-MyD88 KO). Previously generated *Acta2-CreER^{T}* mice [139, 140] expressing CreER^{T} under the α-SMA promoter (Acta-2) were crossed with MyD88$^{fl/fl}$ mice (The Jackson Laboratory). The presence of the transgenes was controlled as previously described by using standard PCR protocols as previously described[48, 140]. Control mice negative for *Acta2*-Cre or Col1α2-Cre but positive for MyD88$^{fl/fl}$ recombination derived from at least five backcrossing with C57BL/6 animals were used in this study in order to have similar microbiota composition. Deletion of MyD88 was induced by intraperitoneal (i.p.) injection of tamoxifen (TMX, 0.5 mg/mice for 4 days, total injection volume/animal is 100 µL). All mice (female, 6-12 weeks of age) were housed under pathogen-free conditions.

Lactobacillus rhamnosus (LbGG) in vivo treatment

Three days after the last tamoxifen or vehicle injection, animals received 5x10^{8} CFU/dose of lyophilized LbGG in suspension in PBS or PBS only for 5 days. Colonic tissue samples were then harvested for further analysis.

Clondronate liposome in vivo treatment for depletion of macrophages

Anionic liposomal clodronate were purchased from FormuMax (Sunnyvale, California, USA). Clondronate liposomes (7 mg/mL) were administered by intraperitoneal injection as previously described by Riehl et al.[124] and per manufacturer's instructions.

Flow cytometry analysis of murine colonic mucosa for macrophage characterization

Five days after the final tamoxifen injection, mice were euthanized and colons were scraped to isolate mucosa. Tissue was digested with 40 µL of collagenase I, II and IV (25000 U/mL stock solutions, Sigma) in 10 mL of HBSS with Ca++& Mg++ (final concentration of each enzyme will be 100 U/ML) at 37°C for 1hr. After run on gentle MACS Dissociator and addition of 50 µL of DNAase stock solution, samples were incubated at 37°C for 30m. Cells were filtered through a 100 µm cell strainer and washed with FACS buffer (0.5 % BSA and 0.02 % NaN3 in DMEM). The cell suspension was centrifuged, resuspended in FACS buffer and cells were counted. Anti-Fc Receptor (anti-CD16/32) antibody (Biolegend) was used to prevent non-specific binding. For staining, 1-2 million cells were incubated with the following antibodies: Cells were stained with the following antibodies: PE-Vio770-conjugated anti-CCR2 (REA538, MACS), BV421-conjugated anti-CX3CR1 (SA011F11, Biolegend), and eFluor660-conjugated anti-F4/80 (BM8, eBiosciences), or F4/80 eFluor 570 (BM8, Thermo Fisher), APC eFluor 780- conjugated anti- CD11b (M1/70, Thermo Fisher), and anti-CD11c. Cells were washed twice, fixed and permeabilized (eBioscience solution) and then incubated with fluorochrome-conjugated antibodies against intracellular antigens including), BV510-conjugated anti-TNF (Mab11, BD), and FITC-conjugated anti-IL-6 (BVREA1034, MACS) for 30 minutes. At the end of the incubation period, cells were washed, then fixed in 0.5 % PFA solution before being analyzed with BD LSRFortessa cell analyzer (Biosciences) per the manufacturer's procedure. Flow cytometry data were analyzed using FACSDiva 6.2 (Becton Dickinson) and FlowJo (Tree Star, USA) software.

Dextran sodium sulfate (DSS) in vivo treatment

Administration of 2% Dextran Sulfate Sodium (DSS) to the drinking water for animals is used to induce epithelial injury. Two days after the final tamoxifen injection (day 4), mice receive 2% DSS for 5 days, to induce acute injury, while control mice receive regular drinking water. On the 5^{th} and 7^{th} day, water supplies were refilled. On day 9 the DSS solution was replaced by regular water. Mice were anesthetized (80 µl of ketamine at 80 mg/Kg and xylazine 10 mg/Kg, i.p.) and euthanized by cervical dislocation.

Histopathological evaluation

Colonic tissue sections were fixed in 4% paraformaldehyde, embedded in paraffin. Assessment of inflammatory score was evaluated by a blinded pathologist specialized in IBD pathology. Hematoxylin and eosin stained sections were evaluated based on the following criteria: destruction of crypts/mucosa, length of colon affected, lymphoycyte infiltration,

disruption of goblet cells, and dysplasia. Each of these criteria was scored from 0 to 3, with 0 absent, 1 slight, 2 moderate and 3 severe, and the inflammatory score was based on the sum of these scores.

Real-time RT-PCR

Analysis was performed according to FastStart TaqMan® Probe Master two-step RT real-time PCR protocol (Roche, Branchburg, NJ). Briefly, all reagents were purchased from Roche Molecular Systems Inc. The appropriate gene expression assay mix for murine beta actin RNA and the gene of interest (a 2x mix of unlabeled PCR primers and TaqMan® MGB probe, FAM™ dye-labeled) and 2 µL of cDNA were added to the PCR reaction step. 20 µL reactions were analyzed with the protocol 2 min at 50°C, 10 min at 95°C (1 cycle) and 15 sec at 95°C and one min at 60°C (40 cycles), using the BioRad CFX96 real time PCR system.

RNA-Seq sequencing

≥ 200ng with RNA Integrity Number (RIN) > 7 of RNA extracted from the murine colonic mucosa were analyzed by RNA-Seq quantification using Illumina NovaSeq and HiSeq platforms with paired-end 150 bp (PE 150) sequencing strategy (Novogene).

Analysis of RNaseq data

Heatmaps were used to demonstrate the expression pattern of differentially expressed genes using CLC Genomics Workbench 12.0.01. Outcomes from RNAseq analysis were uploaded into Ingenuity Pathway Analysis (IPA, QIAGEN) to identify canonical pathways and associated diseases. Venn diagrams of RNAseq canonical pathways were generated using BioVenn.

Statistical analysis

Unless otherwise indicated, the results were expressed as the mean ± SEM of data obtained from at least three independent experiments done with duplicate sets in each experiment. For experiments comparing the results between two groups, Student's *t*-test was used. If necessary, the Wilcoxon signed-ranks test was used. For multiple group comparison, one–

way ANOVA with post-hoc Tukey's Honestly Significant Difference (HSD) test was used. Values of $p < 0.05$ were considered statistically significant.

BIBLIOGRAPHY

1. Lozupone, C.A., et al., *Diversity, stability and resilience of the human gut microbiota.* Nature, 2012. **489**(7415): p. 220-230.
2. Ohland, C.L. and C. Jobin, *Microbial activities and intestinal homeostasis: A delicate balance between health and disease.* Cellular and molecular gastroenterology and hepatology, 2015. **1**(1): p. 28-40.
3. Belkaid, Y. and T.W. Hand, *Role of the microbiota in immunity and inflammation.* Cell, 2014. **157**(1): p. 121-141.
4. Wang, G., et al., *Bridging intestinal immunity and gut microbiota by metabolites.* Cell Mol Life Sci, 2019.
5. Stockinger, B., et al., *The aryl hydrocarbon receptor: multitasking in the immune system.* Annu Rev Immunol, 2014. **32**: p. 403-32.
6. Belkaid, Y. and O.J. Harrison, *Homeostatic Immunity and the Microbiota.* Immunity, 2017. **46**(4): p. 562-576.
7. Kelsen, J.R., P. Russo, and K.E. Sullivan, *Early-Onset Inflammatory Bowel Disease.* Immunol Allergy Clin North Am, 2019. **39**(1): p. 63-79.
8. Meserve, J., et al., *Retrospective Analysis of Safety of Vedolizumab in Patients With Inflammatory Bowel Diseases.* Clin Gastroenterol Hepatol, 2019. **17**(8): p. 1533-1540 e2.
9. Ishige, T., *Growth failure in pediatric onset inflammatory bowel disease: mechanisms, epidemiology, and management.* Transl Pediatr, 2019. **8**(1): p. 16-22.
10. Tripathi, K. and J.D. Feuerstein, *New developments in ulcerative colitis: latest evidence on management, treatment, and maintenance.* Drugs Context, 2019. **8**: p. 212572.
11. Pauwen, N.Y., et al., *Integrated Care for Crohn's Disease: A Plea for the Development of Clinical Decision Support Systems.* J Crohns Colitis, 2018. **12**(12): p. 1499-1504.
12. Siegmund, B., *Medical Therapy of Fibrostenotic Crohn's Disease.* Viszeralmedizin, 2015. **31**(4): p. 259-264.
13. Solberg, I.C., et al., *Clinical Course in Crohn’s Disease: Results of a Norwegian Population-Based Ten-Year Follow-Up Study.* Clinical Gastroenterology and Hepatology, 2007. **5**(12): p. 1430-1438.
14. Sica, G.S. and L. Biancone, *Surgery for inflammatory bowel disease in the era of laparoscopy.* World journal of gastroenterology, 2013. **19**(16): p. 2445-2448.
15. Gordon, I.O., et al., *Fibrosis in ulcerative colitis is directly linked to severity and chronicity of mucosal inflammation.* Alimentary pharmacology & therapeutics, 2018. **47**(7): p. 922-939.
16. Gordon, I.O., et al., *Fibrosis in Ulcerative Colitis: Mechanisms, Features, and Consequences of a Neglected Problem.* Inflammatory Bowel Diseases, 2014. **20**(11): p. 2198-2206.
17. Latella, G. and F. Rieder, *Intestinal fibrosis: ready to be reversed.* Curr Opin Gastroenterol, 2017. **33**(4): p. 239-245.
18. Münch, A., et al., *Undiagnosed microscopic colitis: a hidden cause of chronic diarrhoea and a frequently missed treatment opportunity.* Frontline Gastroenterology, 2019: p. flgastro-2019-101227.
19. Pardi, D.S., *Diagnosis and Management of Microscopic Colitis.* American Journal of Gastroenterology, 2017. **112**(1): p. 78-85.
20. de Mattos, B.R.R., et al., *Inflammatory bowel disease: an overview of immune mechanisms and biological treatments.* Mediators of inflammation, 2015. **2015**.
21. Annese, V., et al., *Impact of new treatments on hospitalisation, surgery, infection, and mortality in IBD: a focus paper by the epidemiology committee of ECCO.* Journal of Crohn's and Colitis, 2015. **10**(2): p. 216-225.

22. Neurath, M.F., *Current and emerging therapeutic targets for IBD.* Nature Reviews Gastroenterology &Amp; Hepatology, 2017. **14**: p. 269.
23. de Souza, H.S.P. and C. Fiocchi, *Immunopathogenesis of IBD: current state of the art.* Nature Reviews Gastroenterology &Amp; Hepatology, 2015. **13**: p. 13.
24. Pastorelli, L., et al., *Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics.* Frontiers in immunology, 2013. **4**: p. 280-280.
25. Sturm, A. and A.U. Dignass, *Epithelial restitution and wound healing in inflammatory bowel disease.* World journal of gastroenterology, 2008. **14**(3): p. 348-353.
26. Iizuka, M. and S. Konno, *Wound healing of intestinal epithelial cells.* World journal of gastroenterology, 2011. **17**(17): p. 2161-2171.
27. Chen, M.L. and M.S. Sundrud, *Cytokine Networks and T-Cell Subsets in Inflammatory Bowel Diseases.* Inflamm Bowel Dis, 2016. **22**(5): p. 1157-67.
28. Jostins, L., et al., *Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease.* Nature, 2012. **491**(7422): p. 119-24.
29. Imam, T., et al., *Effector T Helper Cell Subsets in Inflammatory Bowel Diseases.* Front Immunol, 2018. **9**: p. 1212.
30. Chen, V.L. and D.L. Kasper, *Interactions between the intestinal microbiota and innate lymphoid cells.* Gut microbes, 2014. **5**(1): p. 129-140.
31. Wang, S., et al., *Functions of Macrophages in the Maintenance of Intestinal Homeostasis.* J Immunol Res, 2019. **2019**: p. 1512969.
32. Bain, C.C. and A.M. Mowat, *The monocyte-macrophage axis in the intestine.* Cell Immunol, 2014. **291**(1-2): p. 41-8.
33. Bain, C.C. and A.M. Mowat, *Macrophages in intestinal homeostasis and inflammation.* Immunol Rev, 2014. **260**(1): p. 102-17.
34. Tatano, Y., T. Shimizu, and H. Tomioka, *Unique macrophages different from M1/M2 macrophages inhibit T cell mitogenesis while upregulating Th17 polarization.* Sci Rep, 2014. **4**: p. 4146.
35. Wynn, T.A. and K.M. Vannella, *Macrophages in Tissue Repair, Regeneration, and Fibrosis.* Immunity, 2016. **44**(3): p. 450-462.
36. Quiros, M., et al., *Macrophage-derived IL-10 mediates mucosal repair by epithelial WISP-1 signaling.* The Journal of clinical investigation, 2017. **127**(9): p. 3510-3520.
37. Tortora, G.J. and B.H. Derrickson, *Principles of anatomy and physiology*. 2008: John Wiley & Sons.
38. Saada, J.I., et al., *Subepithelial myofibroblasts are novel nonprofessional APCs in the human colonic mucosa.* J Immunol, 2006. **177**(9): p. 5968-79.
39. Powell, D.W., et al., *Mesenchymal cells of the intestinal lamina propria.* Annual review of physiology, 2011. **73**: p. 213-237.
40. Watts, T.L., et al., *PDGF-AA mediates mesenchymal stromal cell chemotaxis to the head and neck squamous cell carcinoma tumor microenvironment.* J Transl Med, 2016. **14**(1): p. 337.
41. Ijaz, T., et al., *Deletion of NF-kappaB/RelA in Angiotensin II-Sensitive Mesenchymal Cells Blocks Aortic Vascular Inflammation and Abdominal Aortic Aneurysm Formation.* Arterioscler Thromb Vasc Biol, 2017. **37**(10): p. 1881-1890.
42. Pinchuk, I.V., et al., *Intestinal mesenchymal cells.* Curr Gastroenterol Rep, 2010. **12**(5): p. 310-8.
43. Van Vliet, E., et al., *Reticular fibroblasts in peripheral lymphoid organs identified by a monoclonal antibody.* Journal of Histochemistry & Cytochemistry, 1986. **34**(7): p. 883-890.
44. van Vliet, E., M. Melis, and W. Van Ewijk, *Monoclonal antibodies to stromal cell types of the mouse thymus.* European journal of immunology, 1984. **14**(6): p. 524-529.

45. Pinchuk, I.V., et al., *Monocyte chemoattractant protein-1 production by intestinal myofibroblasts in response to staphylococcal enterotoxin a: relevance to staphylococcal enterotoxigenic disease.* J Immunol, 2007. **178**(12): p. 8097-106.
46. Mifflin, R.C., et al., *Intestinal myofibroblasts: targets for stem cell therapy.* Am J Physiol Gastrointest Liver Physiol, 2011. **300**(5): p. G684-96.
47. Pinchuk, I.V., et al., *Stromal cells induce Th17 during Helicobacter pylori infection and in the gastric tumor microenvironment.* PLoS One, 2013. **8**(1): p. e53798.
48. Beswick, E.J., et al., *TLR4 activation enhances the PD-L1-mediated tolerogenic capacity of colonic CD90+ stromal cells.* J Immunol, 2014. **193**(5): p. 2218-29.
49. Mifflin, R.C., et al., *Intestinal myofibroblasts: targets for stem cell therapy.* American journal of physiology. Gastrointestinal and liver physiology, 2011. **300**(5): p. G684-G696.
50. Kurashima, Y., et al., *Mucosal Mesenchymal Cells: Secondary Barrier and Peripheral Educator for the Gut Immune System.* Front Immunol, 2017. **8**: p. 1787.
51. Darby, I.A., et al., *Fibroblasts and myofibroblasts in wound healing.* Clinical, cosmetic and investigational dermatology, 2014. **7**: p. 301-311.
52. Andoh, A., et al., *Role of intestinal subepithelial myofibroblasts in inflammation and regenerative response in the gut.* Pharmacology & Therapeutics, 2007. **114**(1): p. 94-106.
53. Fukata, M., et al., *Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: Role in proliferation and apoptosis in the intestine.* Gastroenterology, 2006. **131**(3): p. 862-877.
54. Zhu, Y., P. Hua, and P. Lance, *Cyclooxygenase-2 Expression and Prostanoid Biogenesis Reflect Clinical Phenotype in Human Colorectal Fibroblast Strains.* Cancer Research, 2003. **63**(2): p. 522-526.
55. Pinchuk, I.V., et al., *Human colonic myofibroblasts promote expansion of CD4+ CD25high Foxp3+ regulatory T cells.* Gastroenterology, 2011. **140**(7): p. 2019-30.
56. Roulis, M., et al., *Intestinal myofibroblast-specific Tpl2-Cox-2-PGE₂ pathway links innate sensing to epithelial homeostasis.* Proceedings of the National Academy of Sciences, 2014. **111**(43): p. E4658-E4667.
57. Brown, S.L., et al., *Myd88-dependent positioning of Ptgs2-expressing stromal cells maintains colonic epithelial proliferation during injury.* J Clin Invest, 2007. **117**(1): p. 258-69.
58. Ciorba, M.A., et al., *Lactobacillus probiotic protects intestinal epithelium from radiation injury in a TLR-2/cyclo-oxygenase-2-dependent manner.* Gut, 2012. **61**(6): p. 829-838.
59. Mifflin, R.C., et al., *Regulation of COX-2 expression in human intestinal myofibroblasts: mechanisms of IL-1-mediated induction.* American journal of physiology. Cell physiology, 2002. **282**(4): p. C824-34.
60. Scaldaferri, F., et al., *Gut Microbial Flora, Prebiotics, and Probiotics in IBD: Their Current Usage and Utility.* BioMed Research International, 2013. **2013**: p. 9.
61. Pinchuk, I.V., et al., *PD-1 ligand expression by human colonic myofibroblasts/fibroblasts regulates CD4+ T-cell activity.* Gastroenterology, 2008. **135**(4): p. 1228-1237, 1237 e1-2.
62. Owens, B.M.J. and A. Simmons, *Intestinal stromal cells in mucosal immunity and homeostasis.* Mucosal Immunology, 2012. **6**: p. 224.
63. Lina, T.T., et al., *Helicobacter pylori cag Pathogenicity Island's Role in B7-H1 Induction and Immune Evasion.* PLOS ONE, 2015. **10**(3): p. e0121841.
64. Beswick, E.J., et al., *Expression of Programmed Death-Ligand 1 by Human Colonic CD90(+) Stromal Cells Differs Between Ulcerative Colitis and Crohn's Disease and Determines Their Capacity to Suppress Th1 Cells.* Front Immunol, 2018. **9**: p. 1125.
65. Hidalgo-Garcia, L., et al., *Can a Conversation Between Mesenchymal Stromal Cells and Macrophages Solve the Crisis in the Inflamed Intestine?* Frontiers in pharmacology, 2018. **9**: p. 179-179.
66. Anderson, P., et al., *CD105 (endoglin)-negative murine mesenchymal stromal cells define a new multipotent subpopulation with distinct differentiation and immunomodulatory capacities.* PloS one, 2013. **8**(10): p. e76979-e76979.

67. Melief, S.M., et al., *Multipotent stromal cells skew monocytes towards an anti-inflammatory function: the link with key immunoregulatory molecules.* Haematologica, 2013. **98**(9): p. e121-e122.
68. Chiossone, L., et al., *Mesenchymal Stromal Cells Induce Peculiar Alternatively Activated Macrophages Capable of Dampening Both Innate and Adaptive Immune Responses.* STEM CELLS, 2016. **34**(7): p. 1909-1921.
69. Gonzalez-Rey, E., et al., *Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis.* Gut, 2009. **58**(7): p. 929-939.
70. Vasandan, A.B., et al., *Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE(2)-dependent mechanism.* Scientific reports, 2016. **6**: p. 38308-38308.
71. Kim, D., et al., *Mesenchymal Cell-Specific MyD88 Signaling Promotes Systemic Dissemination of Salmonella Typhimurium via Inflammatory Monocytes.* Journal of immunology (Baltimore, Md. : 1950), 2017. **199**(4): p. 1362-1371.
72. Wang, L., et al., *Dual functional roles of the MyD88 signaling in colorectal cancer development.* Biomedicine & Pharmacotherapy, 2018. **107**: p. 177-184.
73. Deguine, J. and G.M. Barton, *MyD88: a central player in innate immune signaling.* F1000prime reports, 2014. **6**: p. 97-97.
74. Spiljar, M., D. Merkler, and M. Trajkovski, *The Immune System Bridges the Gut Microbiota with Systemic Energy Homeostasis: Focus on TLRs, Mucosal Barrier, and SCFAs.* 2017. **8**(1353).
75. Menendez, A., et al., *Bacterial Stimulation of the TLR-MyD88 Pathway Modulates the Homeostatic Expression of Ileal Paneth Cell α-Defensins.* Journal of Innate Immunity, 2013. **5**(1): p. 39-49.
76. Su, L., et al., *Development of fatal intestinal inflammation in MyD88 deficient mice co-infected with helminth and bacterial enteropathogens.* PLoS neglected tropical diseases, 2014. **8**(7): p. e2987-e2987.
77. Araki, A., et al., *MyD88-deficient mice develop severe intestinal inflammation in dextran sodium sulfate colitis.* Journal of Gastroenterology, 2005. **40**(1): p. 16-23.
78. Fukata, M., et al., *The myeloid differentiation factor 88 (MyD88) is required for CD4+ T cell effector function in a murine model of inflammatory bowel disease.* Journal of immunology (Baltimore, Md. : 1950), 2008. **180**(3): p. 1886-1894.
79. Fernandes, P., et al., *Differential expression of key regulators of Toll-like receptors in ulcerative colitis and Crohn's disease: a role for Tollip and peroxisome proliferator-activated receptor gamma?* Clinical and experimental immunology, 2016. **183**(3): p. 358-368.
80. Dejani, N.N., et al., *Intestinal host defense outcome is dictated by PGE₂ production during efferocytosis of infected cells.* Proceedings of the National Academy of Sciences, 2018. **115**(36): p. E8469-E8478.
81. Maseda, D., et al., *Nonsteroidal Anti-inflammatory Drugs Alter the Microbiota and Exacerbate Clostridium difficile Colitis while Dysregulating the Inflammatory Response.* mBio, 2019. **10**(1): p. e02282-18.
82. Malvin, N.P., H. Seno, and T.S. Stappenbeck, *Colonic epithelial response to injury requires Myd88 signaling in myeloid cells.* Mucosal immunology, 2012. **5**(2): p. 194-206.
83. Kubinak, J.L., et al., *MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health.* Cell host & microbe, 2015. **17**(2): p. 153-163.
84. Medzhitov, R., et al., *MyD88 Is an Adaptor Protein in the hToll/IL-1 Receptor Family Signaling Pathways.* Molecular Cell, 1998. **2**(2): p. 253-258.
85. Naiki, Y., et al., *MyD88 Is Pivotal for the Early Inflammatory Response and Subsequent Bacterial Clearance and Survival in a Mouse Model of Chlamydia pneumoniae Pneumonia.* Journal of Biological Chemistry, 2005. **280**(32): p. 29242-29249.

86. Asquith, M.J., et al., *Pathogenic and protective roles of MyD88 in leukocytes and epithelial cells in mouse models of inflammatory bowel disease.* Gastroenterology, 2010. **139**(2): p. 519-529.e5292.
87. Frantz, A.L., et al., *Targeted deletion of MyD88 in intestinal epithelial cells results in compromised antibacterial immunity associated with downregulation of polymeric immunoglobulin receptor, mucin-2, and antibacterial peptides.* Mucosal immunology, 2012. **5**(5): p. 501-512.
88. Friedrich, C., et al., *MyD88 signaling in dendritic cells and the intestinal epithelium controls immunity against intestinal infection with C. rodentium.* PLoS pathogens, 2017. **13**(5): p. e1006357-e1006357.
89. Hoshi, N., et al., *MyD88 signalling in colonic mononuclear phagocytes drives colitis in IL-10-deficient mice.* Nature communications, 2012. **3**: p. 1120-1120.
90. Marr, K.A., et al., *Differential role of MyD88 in macrophage-mediated responses to opportunistic fungal pathogens.* Infection and immunity, 2003. **71**(9): p. 5280-5286.
91. Feuerstein, R., et al., *MyD88 in Macrophages Is Critical for Abscess Resolution in Staphylococcal Skin Infection.* The Journal of Immunology, 2015. **194**(6): p. 2735-2745.
92. Lai, J.-F., et al., *Critical Role of Macrophages and Their Activation via MyD88-NFκB Signaling in Lung Innate Immunity to Mycoplasma pneumoniae.* PLOS ONE, 2010. **5**(12): p. e14417.
93. Xiao, E., et al., *Microbiota regulates bone marrow mesenchymal stem cell lineage differentiation and immunomodulation.* Stem Cell Research & Therapy, 2017. **8**(1): p. 213.
94. Manieri, N.A., et al., *Igf2bp1 is required for full induction of Ptgs2 mRNA in colonic mesenchymal stem cells in mice.* Gastroenterology, 2012. **143**(1): p. 110-21 e10.
95. Koliaraki, V., et al., *Innate Sensing through Mesenchymal TLR4/MyD88 Signals Promotes Spontaneous Intestinal Tumorigenesis.* Cell Rep, 2019. **26**(3): p. 536-545 e4.
96. Saada, J.I., et al., *Subepithelial Myofibroblasts are Novel Nonprofessional APCs in the Human Colonic Mucosa.* The Journal of Immunology, 2006. **177**(9): p. 5968-5979.
97. Andréasson, K., et al., *Intestinal dysbiosis is common in systemic sclerosis and associated with gastrointestinal and extraintestinal features of disease.* Arthritis research & therapy, 2016. **18**(1): p. 278-278.
98. Yu, L.C.-H., et al., *Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology.* World journal of gastrointestinal pathophysiology, 2012. **3**(1): p. 27-43.
99. Otte, J.-M., I.M. Rosenberg, and D.K. Podolsky, *Intestinal myofibroblasts in innate immune responses of the intestine.* Gastroenterology, 2003. **124**(7): p. 1866-1878.
100. Mifflin, R.C., et al., *Regulation of COX-2 expression in human intestinal myofibroblasts: mechanisms of IL-1-mediated induction.* American Journal of Physiology-Cell Physiology, 2002. **282**(4): p. C824-C834.
101. Hinz, B., *The role of myofibroblasts in wound healing.* Current Research in Translational Medicine, 2016. **64**(4): p. 171-177.
102. Sun, M., et al., *Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases.* Journal of gastroenterology, 2017. **52**(1): p. 1-8.
103. Alexander, K.L., S.R. Targan, and C.O. Elson, 3rd, *Microbiota activation and regulation of innate and adaptive immunity.* Immunological reviews, 2014. **260**(1): p. 206-220.
104. Neurath, M.F., *Cytokines in inflammatory bowel disease.* Nature Reviews Immunology, 2014. **14**: p. 329.
105. Williams, M.D., C.Y. Ha, and M.A. Ciorba, *Probiotics as therapy in gastroenterology: a study of physician opinions and recommendations.* Journal of clinical gastroenterology, 2010. **44**(9): p. 631-636.
106. Sartor, R.B., *Microbial Influences in Inflammatory Bowel Diseases.* Gastroenterology, 2008. **134**(2): p. 577-594.

107. Floch, M.H., et al., *Recommendations for Probiotic Use—2008.* Journal of Clinical Gastroenterology, 2008. **42**: p. S104-S108.
108. Lin, P.W., et al., *Lactobacillus rhamnosus blocks inflammatory signaling in vivo via reactive oxygen species generation.* Free radical biology & medicine, 2009. **47**(8): p. 1205-1211.
109. Yan, F., et al., *Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth.* Gastroenterology, 2007. **132**(2): p. 562-575.
110. Powell, D.W., et al., *Myofibroblasts. II. Intestinal subepithelial myofibroblasts.* The American journal of physiology, 1999. **277**(2 Pt 1): p. C183-201.
111. Schnoor, M., et al., *Production of Type VI Collagen by Human Macrophages: A New Dimension in Macrophage Functional Heterogeneity.* The Journal of Immunology, 2008. **180**(8): p. 5707-5719.
112. Mäkitalo, L., et al., *Changes in matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinases (TIMP) expression profile in Crohn's disease after immunosuppressive treatment correlate with histological score and calprotectin values.* International Journal of Colorectal Disease, 2009. **24**(10): p. 1157-1167.
113. Uribe, G., et al., *Lactobacillus rhamnosus GG increases cyclooxygenase-2 expression and prostaglandin E2 secretion in colonic myofibroblasts via a MyD88-dependent mechanism during homeostasis.* Cell Microbiol, 2018. **20**(11): p. e12871.
114. Lee, M., et al., *Tissue-specific Role of CX3CR1 Expressing Immune Cells and Their Relationships with Human Disease.* Immune Netw, 2018. **18**(1): p. e5.
115. Cerovic, V., et al., *Intestinal macrophages and dendritic cells: what's the difference?* Trends Immunol, 2014. **35**(6): p. 270-7.
116. Platt, A.M., et al., *An independent subset of TLR expressing CCR2-dependent macrophages promotes colonic inflammation.* J Immunol, 2010. **184**(12): p. 6843-54.
117. Kobayashi, T., et al., *Exclusive increase of CX3CR1+CD28−CD4+ T cells in inflammatory bowel disease and their recruitment as intraepithelial lymphocytes.* Inflammatory Bowel Diseases, 2007. **13**(7): p. 837-846.
118. Kühl, A.A., et al., *Diversity of Intestinal Macrophages in Inflammatory Bowel Diseases.* Frontiers in immunology, 2015. **6**: p. 613-613.
119. Schenk, M., et al., *TREM-1--expressing intestinal macrophages crucially amplify chronic inflammation in experimental colitis and inflammatory bowel diseases.* The Journal of clinical investigation, 2007. **117**(10): p. 3097-3106.
120. Kamada, N., et al., *Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis.* The Journal of clinical investigation, 2008. **118**(6): p. 2269-2280.
121. Adegbola, S.O., et al., *Anti-TNF Therapy in Crohn's Disease.* Int J Mol Sci, 2018. **19**(8).
122. Sartor, R.B., *Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis.* Nat Clin Pract Gastroenterol Hepatol, 2006. **3**(7): p. 390-407.
123. Parameswaran, N. and S. Patial, *Tumor necrosis factor-α signaling in macrophages.* Critical reviews in eukaryotic gene expression, 2010. **20**(2): p. 87-103.
124. Riehl, T.E., et al., *Lactobacillus rhamnosus GG protects the intestinal epithelium from radiation injury through release of lipoteichoic acid, macrophage activation and the migration of mesenchymal stem cells.* Gut, 2019. **68**(6): p. 1003-1013.
125. Eckburg, P.B., et al., *Diversity of the human intestinal microbial flora.* Science (New York, N.Y.), 2005. **308**(5728): p. 1635-1638.
126. Jones-Hall, Y.L. and C.H. Nakatsu, *The Intersection of TNF, IBD and the Microbiome.* Gut microbes, 2016. **7**(1): p. 58-62.
127. Chassaing, B. and A. Darfeuille–Michaud, *The Commensal Microbiota and Enteropathogens in the Pathogenesis of Inflammatory Bowel Diseases.* Gastroenterology, 2011. **140**(6): p. 1720-1728.e3.

128. Villanueva, M.S. and Y. Alimi, *Microscopic colitis (lymphocytic and collagenous), eosinophilic colitis, and celiac disease.* Clinics in colon and rectal surgery, 2015. **28**(2): p. 118-126.
129. van Hemert, S., et al., *Microscopic colitis-microbiome, barrier function and associated diseases.* Annals of translational medicine, 2018. **6**(3): p. 39-39.
130. Khanna;, R.T.T.S.D.P.W.T.S., *New-Onset Microscopic Colitis in an Ulcerative Colitis Patient After Fecal Microbiota Transplantation* American Journal of Gastroenterology., 2006. **111**(5): p. 751-752.
131. Jin, P., et al., *Lactobacillus fermentum ZYL0401 Attenuates Lipopolysaccharide-Induced Hepatic TNF-α Expression and Liver Injury via an IL-10- and PGE2-EP4-Dependent Mechanism.* PloS one, 2015. **10**(5): p. e0126520-e0126520.
132. Kevin J. Tracey, M.D. and P.D. Anthony Cerami, *TUMOR NECROSIS FACTOR: A Pleiotropic Cytokine and Therapuetic Target.* Annual Review of Medicine, 1994. **45**(1): p. 491-503.
133. De Santis, S., et al., *TNFα deficiency results in increased IL-1β in an early onset of spontaneous murine colitis.* Cell Death &Amp; Disease, 2017. **8**: p. e2993.
134. Sica, A. and A. Mantovani, *Macrophage plasticity and polarization: in vivo veritas.* The Journal of clinical investigation, 2012. **122**(3): p. 787-795.
135. Steinbach, E.C. and S.E. Plevy, *The role of macrophages and dendritic cells in the initiation of inflammation in IBD.* Inflammatory bowel diseases, 2014. **20**(1): p. 166-175.
136. Johnson, P., et al., *Isolation of CD 90+ Fibroblast/Myofibroblasts from Human Frozen Gastrointestinal Specimens.* JoVE, 2016(107): p. e53691-e53691.
137. Lepage, G. and C.C. Roy, *Direct transesterification of all classes of lipids in a one-step reaction.* Journal of lipid research, 1986. **27**(1): p. 114-20.
138. Folch, J., M. Lees, and G.H. Sloane Stanley, *A simple method for the isolation and purification of total lipides from animal tissues.* The Journal of biological chemistry, 1957. **226**(1): p. 497-509.
139. Grcevic, D., et al., *In vivo fate mapping identifies mesenchymal progenitor cells.* Stem Cells, 2012. **30**(2): p. 187-96.
140. Worthley, Daniel L., et al., *Gremlin 1 Identifies a Skeletal Stem Cell with Bone, Cartilage, and Reticular Stromal Potential.* Cell, 2015. **160**(1-2): p. 269-284.

CURRICULUM VITAE

NAME: Gabriela Uribe, B.S. Date: July 01, 2019

PRESENT POSITION AND ADDRESS:

Predoctoral Research Fellow (2015-Present)
University of Texas Medical Branch at Galveston
Department of Neuroscience and Cell Biology
Libbie Moody Thompson Basic Science Building
301 University Blvd.
Galveston, TX 77555
Phone (w): (409) 772-7408
Fax: (409) 747-0692
Email: *gauribe@utmb.edu*

BIOGRAPHICAL:

Date of Birth:	December 21, 1990
Birthplace:	Houston, TX
Citizenship:	US Citizen
Languages:	English, Spanish

EDUCATION:

06/2015-present **Doctoral Candidate,**
Field: **Cell Biology**
Graduate School of Biomedical Sciences
Department of Neuroscience, Cell Biology, and Anatomy
The University of Texas Medical Branch, Galveston, TX
Research: MyD88 dependent interactions of myo-/fibroblasts in health and intestinal diseases.

06/2014-06/2015 **Postbaccalaureate Research Education Program (PREP),**
Field: **Microbiology and Immunology**
Department of Microbiology and Immunology
The University of Texas Medical Branch, Galveston, TX
Research: Identification of *Ehrlichia chaffeensis* effector proteins.

08/2009-05/2013 **Bachelors of Science in Biology,**
Field: **Biology**
Department of Biology
The University of St. Thomas, Houston, TX
Research: Phage metagenomics in the Gulf Coast Region.

CERTIFICATIONS:

2014-present Certified in Laboratory Biosafety Level 2, The University of Texas Medical Branch, TX

PROFESSIONAL AND TEACHING EXPERIENCE:

Professional Experience:

06/2015-present **Predoctoral Research Fellow**, Department of Neuroscience, Cell Biology, and Anatomy, University of Texas Medical Branch, Galveston, TX
Mentor: **Dr. Iryna Pinchuk**
Project: Determine how TLR-dependent and independent signaling processes in human colonic myo-/fibroblasts are involved in the initiation of Inflammatory Bowell Disease (IBD) immunopathogenesis and its progression to IBD associated cancer.

06/2014-06/2015 **Graduate Assistant**, Department of Experimental Pathology, University of Texas Medical Branch, Galveston, TX
Mentor: **Dr. Jere W. McBride**
Project: Identification of *Ehrlichia chaffeensis* effector proteins targeted for SUMOylation.

09/2013-06/2014 **QC Technician** Quality Control Department, VGXI, Inc., Houston, TX
Mentor: **Ramu Rameswaran**
Project: Viable and non-viable monitoring of production plant. Microbial plating, gram staining and microbial ID.

09/2011-06/2013 **Student Researcher**, Department of Pathology, Baylor College of Medicine, Houston, TX
Mentor: **Dr. James Versalovic**
Project: Characterized *Bifidobacterium dentium* GadB enzyme and its involvement in small molecule secretions that modulate colonic sensory synapses via stress-induced GABAergic signaling.

06/2010-06/2012 **Undergraduate Researcher** Biology Department, The University of St. Thomas, Houston, TX
Mentor: **Dr. Maia Larios**
Project: Used metagenomic approach to isolate and characterize total DNA from soil samples. Studied Bacteriophage distribution in soil isolated from the Texas Gulf Coast region.

06/2010-06/2012 **Biology Lab TA** Biology Department, The University of St. Thomas, Houston, TX
Mentor: **Jonathan Newsome**
Project: Maintain lab equipment, assist students, and grade quizzes.

Training and Supervision of Undergraduate, Graduate and Medical Students

05/2018-07/2018 Thomas Alterman, The University of Texas Medical Branch

10/2016-12/2017 Russel Rourke, Medical Resident, The University of Texas Medical Branch

05/2016-02/2017 Rachel Dillard, Medical student, The University of Texas Medical Branch

06/2012-12/2012 Reyes, Pedro, Medina, Nicole, Hernandez, Angela, Undergraduate students, The University of St. Thomas

RESEARCH ACTIVITIES:

Area of Research

Gabriela Uribe is currently a PhD candidate in the Cell Biology Graduate Program at The University of Texas Medical Branch (UTMB) in Galveston. Her research interests are in the exploration of cellular, molecular pathobiology, and immunology. The objective of her research project and part of her PhD dissertation project is to determine how TLR-dependent and independent signaling processes in human colonic (myo)fibroblasts are involved in the initiation of Inflammatory Bowel Disease (IBD) and its progression to IBD associated cancer. While working to this goal she will acquire extensive research skills in cell biology, molecular biology, microbiology, and immunology. Gabriela Uribe has substantial knowledge in the area of host-microbe interactions and over 6 years of experience in microbiology and cell biology.

Previous Research

Animal Care: Handle and restrain; Perform necropsy; Tissue collection.

Cell Culture: Tissue and Cell Culture; Mycoplasma Testing; Infection (*E. Chaffeensis*); Growth of primary cell lines; Primary tissue processing.

Clinical: Bench-level bacteriology, virology, serology, mycology, mycobacteriology and parasitology; Plate rounds; Member of Diagnostic Management Team; Pathlology Grand Rounds; Member of Gut Inflammation Multidisciplinary Translational Teams.

Computer Skills: Microsoft Office, Image J, Vector NTI, BLAST, GraphPad, MxPro, PyMOL; Sequence analysis; Data processing; Statistical analysis; Computer languages (R.).

Microbiology: USDA Soil sampling; Aseptic technique; Anaerobic techniques; Plate counting; Growth phase analysis; Prepare media; Pour plates; Plasmid amplification.

Microscopy: White Light; Confocal; Immunofluorescence Immunohistochemistry.

Molecular Biology: Cloning; DNA and RNA isolation; ELISA; Western Blotting; SDS-PAGE; Electrophoresis; Real-Time PCR; Transfection; Transformation; Electroporation; Primer Design; Site-Directed Mutagenesis; RNAi (*C. elegans*); Affinity protein purification; Analyze peptides; Manipulate DNA; Mutagenesis; Flow cytometry.

General Laboratory: Pipette; Weigh; Sterilize; Filtrate; Titrate; Wash/Clean glassware; Prepare media; Practice sterile techniques

Laboratory/Research Instruments: pH meter; Centrifuges: standard, high speed; Incubators; Dark room equipment; Volumetric glassware; Microtome.

Quality Control: Viable and non-viable monitoring of production plant; Microbial plating, gram staining and microbial ID; Writing SOPs; CGMP training.

Training: Collaborative Institutional Training Initiative (CITI) for protection of human subjects; American Association for Laboratory Animal Science (AALAS) for animal users.

Grant Support

Current

5TL1TR001440-03 (Trainee: Gabriela Uribe) ` 1/16/2018-1/16/2019

CTSA/TL1 Predoctoral Trainee Award

Myo-/fibroblast My D88 Dependent Signaling in Epithelial Barrier and Fibrosis.

The objective of my research project and part of my PhD dissertation project is to determine how Toll-Like Receptor (TLR)-dependent and independent MyD88 signaling processes in human colonic myo-/fibroblasts are involved in maintaining colonic homeostasis. Additionally, I want to see how disruption of these signaling processes leads to the initiation of Inflammatory Bowell Diseases (IBD).

MEMBERSHIPS IN SCIENTIFIC SOCIETIES:

National:

11/2017-present	Associate Member, American Association for Cancer Research
02/2017-present	Student Member, American Gastroenterological Association
08/2015-present	Student Member, American Society for Microbiology

Local:

01/2018-12/2018	Regular Member, Southwestern Association of Clinical Microbiology
08/2015-to present	Student Member, Society for Cell Biology
08/2015-present	Student Member, Society for Cell Biology

HONORS AND AWARDS

08/2012	Cullen Endowed General Scholarship, The University of St. Thomas
08/2011	HSI STEM stipend, The University of St. Thomas
08/2010	Prince of Peace Parish Scholarship, The University of St. Thomas
06/2010	HSI STEM stipend, The University of St. Thomas
08/2009	Francis E. Monaghan Fulltime scholarship, The University of St. Thomas
02/2017	Oral presentation at Digestive Disease Week (among the top 5 percent of abstracts submitted), Chicago, IL, May 06-09, 2017. Title: *Lactobacillus rhamnosus* GG stimulates COX-2-derived PGE_2 predominantly in colonic (myo)fibroblasts via a MyD88 dependent mechanism.
05/2017	AGA Early Career Investigator Award
03/2018	CTSA/TL-1 Training Award
05/2018	AACR Minority Scholar in Cancer Research Awards
12/2018	Shirley Patricia Parker Scholarship Endowment in the Graduate School of Biomedical Sciences
12/2018	The Arthur V. Simmang Scholarship Fund
05/2019	Oral presentation at Digestive Disease Week

COMMUNITY SERVICE AND ACTIVITIES:

09/2012-12/2012 Volunteer at Montrose Counseling Center

02/2010-05/2010 Volunteer at New Era Nursing Home

07/2011-07/2011 Mission trip to Guatemala with Prince of Peace Church

PUBLISHED:

A. Articles in Peer-Reviewed Journals:

1. **Uribe, G.,** Villéger, R., Bressollier, P., Worthley, D., Wang, T., Powell, D. W., Urdaci, M. C., **Pinchuk, I.V**. *Lactobacillus rhamnosus* GG increases cyclooxygenase-2 expression and prostaglandin E2 secretion in colonic myofibroblasts via a MyD88-dependent mechanism during homeostasis. *Cellular Microbiology*. 2018;e12871. https://doi.org/10.1111/cmi.12871
2. Pokusaeva K, Johnson C, Luk B, **Uribe G**, Fu Y, Oezguen N, Matsunami RK, Lugo M, Major A, Mori-Akiyama Y, Hollister EB, Dann SM, Shi XZ, Engler DA, Savidge T, Versalovic J. (2017). GABA-producing Bifidobacterium Dentium Modulates Visceral Sensitivity in the Intestine. Neurogastroenterology and Motility, Jan; 29(1): e12904. PMCID: PMC5195897

B. Selected abstracts presented and published over the last seven years (2010-2017):

1. **Uribe, G**., Khanipov, K., Golovko, G., Villeger, R., Grim, C., He, J., Beswick, E.B., Powell, D.W., Pinchuk, I.V. Myo-/Fibroblast MyD88-Mediated Signaling Regulates Inflammatory Responses in the Colon by Suppressing Influx of Inflammatory Macrophages. Federation of Clinical Immunology Societies. Boston, MA. June 18-21,2019.
2. **Uribe, G**., Khanipov, K., Golovko, G., Villeger, R., Grim, C., He, J., Beswick, E.B., Powell, D.W., Pinchuk, I.V**.**Colonic stromal cell MyD88-mediated signaling regulates inflammatory responses in the colon by suppressing influx of inflammatory macrophages.Digestive Disease Week. San Diego, CA. May18-22, 2019.
3. **Uribe, G**, Loeffelholz, M, Bufton, K, Williiams-Bouyer, N. Evaluation of C. Diff Quick Chek Complete; comparison with Xpert C. diff PCR test. Southwestern Association of Clinical Microbiology. San Antonio, TX, September 5-8, 2018.
4. **Uribe, G.**; Rourke R.; Villeger R.; Golovko G.; Khanipov K.; Liu Z.; Pimenova M.; Fofanov Y.; Zhou J.; Brasier A.R.; Pinchuk I.V. *Escherichia coli* induces tumor-promoting inflammatory cytokine IL-6 in cancer associated fibroblasts (CAFs) in a NF-κB/BRD4 dependent manner. American Association for Cancer Research, Chicago, IL, April 14-18, 2018.
5. **Uribe, G**.; Villeger, R.; Dillard, R; Powell, D.W.; Urdaci, M.C.; Pinchuk, I.V. *Lactobacillus rhamnosus* GG stimulates COX-2-derived PGE_2 predominantly in colonic (myo)fibroblasts via a MyD88 dependent mechanism. Digestive Disease Week, Chicago, IL, May 6-9, 2017.
6. Villeger, R., **Uribe, G**., Trieu, J.A., Johnson ,P., Qiu, S., Don W. Powell, D.W., Beswick E.J., Pinchuk, I.V. $Adh1b^{low}/Cyp26b1^{high}$ activity of $CD90^{+}$ (myo)fibroblasts supports tumor-promoting inflammation in colorectal cancer. American Association for Cancer Research, Washington, DC, April 1 - 5, 2017.

7. **Uribe G**., Farris, T.R, Zhang, X., and Jere W. McBride **.**Characterization of novel *Ehrlichia chaffeensis* SUMOylated effector proteins. Summer 2015 General Meeting of the American Society for Microbiology, New Orleans, LA, May 30-02, 2015.
8. **Uribe G**., Farris, T.R, Zhang, X., and Jere W. McBride **.**Characterization of novel *Ehrlichia chaffeensis* SUMOylated effector proteins Department of Pathology's Trainee Research Day, Galveston, TX, April , 2015.
9. **Uribe G.**, Farris, T.R, Zhang, X., and Jere W. McBride. Identification of *Ehrlichia chaffeensis* effector proteins targeted for SUMOylation. Fall 2014 General Meeting of the American Society for Microbiology, Houston, TX, November 06-08, 2014; Abstract 44
10. **Uribe G.,** Characterization of Glutamate Decarboxylase System in the Intestinal Commensal Bacterium *Bifidobacterium dentium* ATCC 27678. Spring 2013 University of St. Thomas Research Symposium, Houston, TX.
11. Brown, S., Andrade, D., **Uribe, G.,** McCleskey, S. , Ticas, D., Zaibaq, J., Griffin, R., Sen, P., Jain, R., Simmons, A., Frohlich, D., Rosell, R., McWhinney, D., and Larios, M. Isolation and Characterization of Bacterial Phage: Metagenomics Study of Phage Population from Texas Gulf Coast Region. American Society for Microbiology (ASM) Regional Meeting, San Marcos, TX., October 28-30, 2010.
12. Brown, S., Andrade, D., **Uribe, G.,** McCleskey, S. , Ticas, D., Zaibaq, J., Griffin, R., Sen, P., Jain, R., Simmons, A., Frohlich, D., Rosell, R., McWhinney, D., and Larios, M. Isolation and Characterization of Bacterial Phage: Metagenomics Study of Phage Population from Texas Gulf Coast Region. Summer 2010 *CCRAAHSI Research Conference*, Houston, TX, August, 2010.

C. Articles Submitted/Pending under revision to peer-reviewed journals

1. E. Aguirre, E.J. Beswick, C. Grim, **G. Uribe**, R. Villeger, J.M. Starkey, Y. Cong, D.W. Powell, **I.V. Pinchuk**. Increased Matrix Metalloproteinases in Crohn's Disease mediate cleavage of membrane bound PD-L1 on (myo)fibroblast which regulate T helper cell responses. *Cellular and Molecular Immunology*. **2018**. *Submitted, under review*.

This dissertation was typed by Gabriela Uribe, B.S.