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The mechanism and potential therapeutic effects of Cyclophilin and Syndecan 1 in Inflammatory Bowel Disease: Can this combination treatment duplicate the anti-inflammatory effect of Cyclosporine without its immunosuppressive effect?

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THE MECHANISM AND POTENTIAL THERAPEUTIC EFFECTS OF CYCLOPHILIN AND SYNDECAN 1 IN INFLAMMATORY BOWEL DISEASE:

CAN THIS COMBINATION TREATMENT DUPLICATE THE ANTIINFLAMMATORY EFFECT OF CYCLOSPORINE WITHOUT ITS IMMUNOSUPPRESSIVE EFFECT?

by

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Today is the day, after a compressed three-year time period. Not only scientifically, but also personally, it has been a really rewarding experience. It was difficult to manage my academic and research work with being a mother, a housewife, and a working mother during this time. But I am glad to report that with my unlimited commitment, perseverance, good time management and supportive environment, I prevailed.

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ABSTRACT

Thesis Title: The mechanism and potential therapeutic effects of Cyclophilin and Syndecan 1 in Inflammatory Bowel Disease: Can this combination treatment duplicate the anti-inflammatory effect of Cyclosporine without its immunosuppressive effect?

Background: Inflammatory Bowel Diseases constitute a group of refractory autoimmune disorders with inflammation and genetic susceptibility being the underlying basis of their pathogenesis. Their treatment modalities have evolved over the years to include immunoregulators like cyclosporine A, an immunosuppressant that interacts with cytoplasmic cyclophilin A, in addition to probiotics to correct dysbiosis. However, several complications followed such protocols.

Aims: This study aimed to explore and compare the possible role of Syndecan-1 knock out mice compared to wild type Balb/c mice in the IBD pathogenic process as well as the effectiveness of cyclophilin A and cyclosporine A in the management of IBD in presence of probiotics.

Materials and Methods: IBD was induced in a total of 112 mice equally divided between Syndecan-1 knock out and Balb/c wild type mice, using 2% Dextran Sulfate Sodium (DSS) for a week followed by 2 weeks of treatment with 200µg every other day cyclosporine A and 25µg per day cyclophilin A, intraperitoneally. In addition, a daily dose of 10^8CFU of a combination of probiotics in drinking water.

Animals were monitored for clinical signs and symptoms and checked for gross pathologies in the abdomen on the sacrifice day. Descending colon and sigmoid biopsies were removed and fixed, for routine microscopy as well as frozen for protein extraction and molecular testing of IL-6, CD3, CD147, Beta-1 integrins as well as pAkt expression.

Results: Data from the study showed that, in general, the induction of IBD in the Syndecan-1 knock out mice was more sever at clinical, histologic and molecular levels than in the wild type with a higher Daily Activity Index (DAI) and more sever intestinal histological alterations. Separate treatment with cyclophilin A or cyclosporine A showed more inhibition of IL-6 in the wild type but no added inhibitory effect when administered as a combination together. Probiotics added to the combination was more effective in the wild type and when used alone its inhibition of IL-6 was the highest especially in the wild type.

As for the CD147 marker there were more suppressions across the various groups in the knock out mice except for the probiotics alone which made no major difference between the 2 strains. Concerning CD3, the expression in the DSS alone group was much more in the KO mice 7.45 compared to 4.25 ratios over controls. Cyclophilin A decreased CD147 expression more in the KO than the wild type. There was a major suppression of CD147 with CyA in the KO mice much more than the wild type. On the other hand, the combination led to a rise in expression of CD147 compared to either CyA or CypA alone in the KO mice, an exacerbation. However, when probiotics was added to the combination, it had little if any effect in the KO, but a major effect in wild type, in decreasing expression. On the other hand, probiotics alone significantly decreased expression of CD147 in the KO mice as well as wild type As for CD3, it was twice as much elevated in the KO mice by DSS treatment and suppressed more by CyA. However, the CypA-CyA complex increased significantly CD3 expression and led to more inflammation in knock out mice. Moreover, the probiotics had little effect with the combination by a major effect alone in the KO by decreasing the expression of CD3.

In relation to Beta-1 integrins, DSS led to more expression in the wild type compared to KO and CypA had less effect in decreasing this expression in KO contrary to the CyA which led to more expression in KO. The combination made no significant difference from CyA alone and had no added value. However, adding probiotics increased the expression to almost double in KO, exacerbation. Probiotics used alone had little effect on Beta-1 integrin expression but a significant effect in reducing expression in wild type. As for pAkt, it was very well expressed and upregulated in both strains treated with DSS, but much more in the KO mice compared to controls without DSS. Separate CypA and CyA treatments brought the levels close to those in the respective controls, i.e. still higher in KO. The complex CypA-CyA showed an added effect of both drugs. Probiotics in the complex had similar relative reduction effects in both strains while probiotics alone led to further reduction of pAkt expressions in both strains but more so in the KO.

Conclusion: In comparison to the wild type, the inflammation was more sever in the KO mice, and treatment with CyA led to more reduction in inflammation and more suppression of expression of immune cell markers. In general, CypA also inhibited expression of inflammation markers more in KO mice. However, the combination of both CypA-CyA caused exacerbation of inflammation in the KO and not in the wild type. On the other hand, when probiotics were added to the CypA-CyA, they had little effect in KO but more effect in the wild type to decrease inflammation. Probiotics used alone ameliorated the inflammation in both strains significantly more in wild type in most cases. The differential effects of CyA, CypA, probiotics and their combinations on the various inflammatory markers as well as the histological alterations and clinical signs and symptoms speak in favor of a possible role of Syndecan-1 and is still using CyA in IBD treatment without adding extra cyclophilins. However, probiotics need to be considered

after more explorations on the mechanisms involved in presence of CypA and CyA especially that pAkt was less active in their presence.

Keywords: Cyclosporine A, Cyclophilin A, Probiotics, IBD, inflammation, Syndecan-1

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List of Abbreviations

	A
AMPs	Antimicrobial Peptides
ANOVA	Analysis of variance
	B
bFGF	Basic Fibroblast Growth Factor
BFM	Bifidobacterium-Fermented Milk
CAMs	Several Adhesion Molecules
CAT	Catalase
CAT	Catalase Enzyme
CD	Chron's Disease
CD3	Cluster of Differentiation 3
COXs	Cyclooxygenases
CRC	Colorectal Cancer
CYA	Cyclosporine A
CYPA	Cyclophilin A
	C
DAI index	Daily Activity Index
DAPI	4',6-diamidino-2-phenylindole
DHE	Dihydroethdium
DNBS	Dinitrobenzene Sulfonic Acid
DSS	Dextran Sulfate Sodium

	E
EMMPRIN	Extracellular Matrix Metalloproteinase Inducer
EMT	Epithelial-Mesenchymal Transition
ENS	Enteric Nervous System
ER	Endoplasmic Reticulum
	F
FAK	Focal Adhesion Kinase
	G
GM-CSF	Granulocyte Macrophage Colony stimulating Factor
GPXs	Glutathione Peroxidases
GSH	Glutathione Peroxidases
GWAS	Genome-Wide Association Studies
	H
H&E	Hematoxylin and Eosin
HFD	High Fat Diet
HPA	Hypothalamic-Pituitary-Adrenal
HS	Heparan Sulfate
HSPGs	Heparan Sulfate Proteoglycans
	I
IBD	Inflammatory Bowel Disease
ICAM	Intercellular Adhesion Molecule
ICAM-1	Intercellular Adhesion Molecule 1
IECs	Intestinal Epithelial Cells
iNOS	Inducible Nitric Oxide Synthase

	J
JNK	Jun N-Terminal Kinase
	K
KO	Knockout
	L
LCN-2	Lipocalin 2
LOXs	Lipoxygenases
	M
Mad-	
CAM-1	Mucosal Addressing Cell Adhesion Molecule 1
MDP	Muramyl Peptide
MDP	Muramyl Dipeptide
MEL	Mannosylerythritol Lipid
MFI	Mean Fluorescence Intensity
MNPs	Mononuclear Phagocytes
MPO	Myeloperoxidase
MYLK	Myosin Light Chain Kinase
	N
NFAT	Activated T-Lymphocytes
NKTs	Natural Killer Cells
NOD2	Nucleotide-Binding Oligomerization Domaincontaining 2
NOS	Nitric Oxide Synthase
NOXO1	Nadph Oxidase Organizer 1
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs

	P
PAF	Platelet Activating Factor
PAMPs	Pathogen-Associated Molecular Patterns
PMNs	Polymorphonuclear Leukocytes
PRRs	Pattern Recognition Receptors
PSC	Primary Sclerosing Cholangitis
PUFA	Polyunsaturated Fatty Acids
PUMA	P53-Upregulated Modulator Of Apoptosis
	R
RA	Rheumatoid Arthritis
RIPK2	Receptor- Interacting Serine/Threonine-Protein Kinase 2
ROI	Region Of Interest
ROS	Reactive Oxygen Species
	S
SCFAs	Short-Chain Fatty Acids
SCID	Severe Combined Immune Deficiency
SLE	Systemic Lupus Erythematosus
SLE	Systemic Lupus Erythematosus
SNPs	Nucleotide Polymorphisms
SOD	Superoxide
SOD	Superoxide Dismutase
	T
TAMs	Tumor-Associated Macrophages
TBST	Tris-Buffered Saline and Tween

TCR	T-Cell Receptor
TGF-ß	Transforming Growth Factor- ßeta
TIMP-1	Matrix Metalloproteinase
TL1A	Tnf-Like Molecule 1a
TLRs	Toll-Like Receptors
TLRs	Toll-Like Receptors
TNBS	Trinitrobenzene Sulfonic Acid
TNF-α	Tumor Necrosis Factor-alpha
Treg	T-Regulatory-1
	U
UC	Ulcerative Colitis
	V
VCAM1	Vascular Cell Adhesion Molecule 1

CHAPTER I
INTRODUCTION

1. Preamble

Inflammatory Bowel Diseases (IBD's) constitute a panel of refractory autoimmune inflammatory disorders ranging from ulcerative colitis (UC) to Chron's disease (CD) and others [1]. The basic underlying mechanism of the disorders is inflammation, a crucial process to restore homeostasis and maintain a normal physiological status in the body [2].

The exact etiology of IBD is not fully elucidated, although studies have documented that IBD is a result of a plethora of genetic and non-genetic environmental interactions involving the microbiome, the environment and the immune system, which plays a crucial role in initiating an excessive inflammatory response in genetically susceptible individuals [3]. Consequently, a panoply of proinflammatory and anti-inflammatory secretions interplay through various signaling pathways to reestablish homeostasis. The microbiome, whether dysbiotic or eubiotic, is an essential player in such pathogenic processes as demonstrated in multiple human and animal studies, in our laboratory and other centers for the past 2 decades or more [4-6].

The treatment modalities of IBD's have deeply changed over recent years following discoveries of the multiple pathogenic pathways involved and accordingly treatment targets in IBD have also evolved stressing healing rather than symptoms control. On this basis, various management protocols of IBD's included immunoregulators like cyclosporine or anti cytokines antibodies like anti TNF-α

and anti-IL-23, or antiinflammatory compounds like salycilates among others [7]. In addition, the approach of using probiotics to balance the dysbiosis has been adopted in multiple instances with encouraging results, both in animal and human studies [8, 9].

Literature reported that each of the adopted management protocols has not been without limitations and drawbacks. Cyclosporine A, the immunosuppressant exhibited several complications in terms of toxicity to various body organs. It was believed that the administration of cyclophilin A (CYPA) compound with properties of interacting with cyclosporine (CYA) would enhance its immunosuppressant effect and probably decrease cyclosporine side effects. On such premises, this study was undertaken to explore the beneficial effect of CYA and CYPA for IBD management, particularly in the presence of probiotics. In addition, it is believed that data emanating from the present study could highlight a mechanism by which CypA-CyA combination could treat IBD by immunosuppressing the autoimmune reaction with less side effects in the Syndecan 1 null (Synd-1 KO) mice. This approach is also supported by data that polymorphisms in IBD susceptibility genes in various strains including Syndecan-1 knock out mice, affect gut microbiota and disrupt the homeostasis of microbes internally, rising likelihood of IBD development.

Based on the genetic variability of IBD's, the above hypothesis was broadened to include the effect of Syndecan-1, a protein essential for epithelial healing, by inducing a DSS-IBD in Syndecan1knock out mice and compare the course of IBD to wild type animals undergoing similar treatment protocols. Such an approach to

IBD will possibly pave the way to the understanding of several mechanistic pathways involved in maintaining intestinal homeostasis.

Based on this background, this study aimed at exploring (1) the potential difference in DSS-IBD induction between various mice strains and the possible role of Syndecan-1 knock out mice in such a process, (2) the effectiveness of cyclosporine A in the management of IBD and its multiple side effects, and (3) the potential role of cyclophilin A in reducing such effects and consequently enhancing the heeling process of the inflamed gut. We undertook this project aiming also to explore the potential role of probiotics in IBDs in a Syndecan-1 knock out mice model compared to the wild type.

Data resulting from this study shed light on the potential mechanisms involved in the pathogenesis of IBD and lay the ground for a novel approach to its management. Such data would also open the road for further in-depth exploration for the signaling pathways involved and the, so far, unfolded mechanistics of pathogenesis, the basis for new therapeutic modalities.

Comparing the wild type to Syndecan-1 knock out mice could open the road for further and in-depth exploration of new therapeutic modalities for IBD.

2. General Characteristics of IBD:

IBDs are a group of inflammatory autoimmune disorders that include Chron's Disease (CD), Ulcerative Colitis (UC), and an array of others. Inflammation is the primary underlying mechanism of IBD. The immune system responds quickly to external aggressions in order to protect itself against invaders like bacteria and diseases; this action is referred to as "inflammation."[2, 10]. In order to maintain homeostasis and a proper physiological state in the body, inflammation is a vital

process. The gastrointestinal system normally performs a number of physiological processes, including food absorption and metabolism as well as ion and waste product secretion. As a result, in addition to a variety of commensal microorganisms, the alimentary canal is primarily exposed to diverse sources of antigens found in meals. In addition to being an essential physical barrier, mucosal epithelial cells also secrete a variety of antimicrobial defense chemicals, including cathelicidins, defensins, and bacteriostatic proteins like lipocalin 2 (LCN-2), as well as mucins and cytokines [11-13]. Additionally, distinct immune cells found in the lamina propria or in various organized structures, such as mesenteric lymph nodes and Peyer's patches, are necessary for the mucosal immune response [14]. This reaction is influenced by both hereditary and environmental variables. An influx of neutrophils and macrophages, proteolytic enzymes, and free radicals are therefore released when intestinal homeostasis is disturbed [15, 16], resulting in either acute or chronic intestinal inflammation, like inflammatory bowel disease (IBD).

IBD is a chronic relapsing disorder that is primarily marked by the existence of uncontrolled intestinal inflammation and epithelial injury in the gastrointestinal tract [3, 17-20]. Episodes of abdominal pain, diarrhea, bloody stools, severe rectal bleeding, weight loss, fever, and fatigue are among the most typical signs and symptoms of IBD. [15, 21-23]. IBD is recognized to have a multifactorial etiology, meaning that both genetic and environmental variables have an impact on how the disease develops and progresses. Epithelial barrier deficiencies, environmental variables, dysregulated immunological responses, and disruption of the gut microbiota are among the non-genetic contributing factors [23-29].

The major characteristic of Crohn's disease (CD) is transmural inflammation, which can discontinuously develop in any area of the GI tract in a discontinuous pattern, beginning in the mouth and terminating in the anus. However, it is generally restricted to the colon, perianal region, cecum, and terminal ileum. Histologically, it is associated with the existence of granulomas, fissuring ulcerations, and thickened submucosa. In CD, skip lesions, cobble stoning, and strictures have also been detected by colonoscopy. While pancolitis can affect the entire colon or a portion of it in a continuous pattern, inflammation in UC primarily affects the rectum and sigmoid colon. The submucosa and mucosa, where cryptitis and crypt abscesses are common histological findings in UC, are the only areas of this superficial inflammation. Additionally, colonoscopy has revealed the presence of persistent inflammatory regions and pseudo polyps [21, 30-37]. Moreover, researchers demonstrated that indeterminant colitis patients, meaning that their disease symptoms are not typical for either UC or CD, constitute 10 % of IBD patients. Unfortunately, they have the potential to develop one of these diseases down the road as the disease worsens [20].

3. Epidemiology and Risk Factors:

A systematic review has indicated that from the middle of the 20th century, the incidence of ulcerative colitis and Crohn's disease has increased throughout the Western world, including Europe, North America, New Zealand, and Australia. [38]. According to studies, 2.5 million people in Europe and 1.5 million people in North America have IBD, and 0.4% of those populations live with the disease [39, 40]. On the other hand, it was shown that in South America, Asia, and the Middle East this incidence was very low during the 20^{th} century [41-46]. Although the

incidence rate of IBD in those nations began to increase [38]. Geographic areas have an impact on the disease's incidence [47]. As an illustration, Western Europe has the highest incidence rate whereas Mediterranean neighboring nations have the lowest [48, 49]. Nowadays, IBD prevalence is increasing as well as its incidence over the world, particularly in South America and Asia [50]. Additionally, it was noted that people are more likely to develop IBD when they move to nations where the condition is prevalent [51]. Moreover, research has shown that IBD can occur at any age, but the majority of people who are affected are between the ages of 15 and 29 [52]. According to additional studies, 25 percent of cases are diagnosed in children or teenagers. [53, 54].

A population-based case control study has revealed that both men and women experience the same incidence of IBD. However, both race and ethnicity have an impact on it. For instance, the risk of IBD is threefold increase in Jewish people than in nonJewish. [55]. Additionally, it has been noted that people with IBD are more likely to have cardiovascular problems, infections and cancer-related mortality, particularly colorectal cancer (CRC) [56, 57]. It is believed that CRC accounts for 10–15% of the mortality in

IBD patients [58].

3.1 Genetics:

Although studies have shown that IBD is caused by a variety of genetic and nongenetic interactions, involving the microbiome, the environment, and the immune system, which plays a critical role in initiating an excessive inflammatory response against the host microbiome in genetically susceptible individuals, the exact etiology of IBD is still unknown and not fully elucidated. [3, 59, 60]. Genome-wide association studies (GWAS), next-generation sequencing studies,

and other analyses have shown about 240 non-overlapping genetic risk loci, with about 30 genetic loci shared by CD and UC [61- 63]. Both ulcerative colitis and Crohn's disease are polygenic diseases. Moreover, susceptibility sites on 12 chromosomes were discovered by 12 genome-wide scans. The genetic heterogeneity of inflammatory bowel disease is consistent with the fact that no single locus has been consistently found in all genome scans. IBD1-9 now refers to the regions on chromosomes 1, 3, 5, 6, 12, 14, 16, and 19. Using positional cloning methods and detailed mapping of susceptibility regions identified by full genome scans, several genes have recently been found [64]. It is worth noting that two thorough meta-analyses indicated that the major histocompatibility complex gene, located on chromosome 6 (IBD3), provides the strongest evidence for a link to inflammatory bowel disease across all populations and diseases [65-67]. Despite having diverse effects, more than 50% of IBD-related genes have been linked to other autoimmune illnesses [68]. Even though UC and CD have different clinical characteristics, both diseases share about 70% of IBD related genes [69].

Numerous loci comprising genes, including MST1, IL2, CARD9 and REL, have been identified to exist between ulcerative colitis and the associated consequence primary sclerosing cholangitis (PSC) [70]. Finding UC individuals who are at risk for other diseases like PSC requires careful consideration of this overlap. Similar to this, a number of genes, including NOD2, C13orf31 (FAMIN), and LRRK2 are shared by Mycobacterium leprae infection and Crohn's disease [70].

A few pathways involved in preserving intestinal homeostasis, including cell migration, immunological control, epithelial barrier function, innate mucosal

defense, autophagy, and adaptive immunity, have become better understood as a result of research on the genes associated with IBD [35, 71-73]. Several studies have shown that relatives of IBD patients had a higher risk of developing the condition than the general population, supporting the idea that the pathophysiology of the illness is closely correlated with genetic factors [74]. According to studies, 12% of those who are afflicted have a history of IBD in their families [75-77]. Additionally, changes in the gut microbiota and disruption of crucial host-microbe interactions caused by mutations in IBD susceptibility genes increase the risk of IBD development. These genes may be used as genetic biomarkers to identify individuals who are more likely to experience IBD, enabling early diagnosis and treatment. GWAS has found a number of strongly related susceptibility genes, although they only make up around 25% of the estimated heritability [78].

A cytosolic protein called NOD2 (nucleotide-binding oligomerization domaincontaining 2), which is found in monocytes, macrophages, intestinal epithelial cells including Paneth cells, and lamina propria lymphocytes including T cells, codes for an intracellular pattern recognition receptor that is essential for the host-microbe immunological response. The NOD2 gene, which is found on chromosome "16," has been linked to IBD, particularly CD [79-85]. Normally, it binds to the muramyl peptide (MDP), a conserved motif present in the bacterial cell wall peptidoglycan [86]. Accordingly, NFKB and MAPK are activated and thus inflammatory cytokines (e.g., TNF and IL-1β) and antimicrobial peptides are transcribed [85, 87-89]. It should be noted that the chronic NOD2 stimulation causes a defect in all the NOD2-mediated mechanisms that downregulate inflammation in the carriers of the genetic variants responsible for NOD2 mutations

[90]. Knowing that NOD2 is normally expressed in intestinal epithelium, its mutations subsequently lead to a defect in the components of an intact mucus layer, resulting in a disrupted colonic mucosal barrier with a reduced antimicrobial peptide secretion and increase of pathogenic bacteria [91]. In addition, the impairment of NOD2 contributes to an alteration in the gut microbiome, and thus a disruption in the gut homeostasis, leading to dysbiosis and inflammation, and hence increasing the risk of IBD [92, 93]. Additionally, NOD2 has also been involved in the autophagy process [35, 94]. Upon MDP stimulation, autophagy is activated, and thus the intracellular bacteria are confined within autophagosomes, leading further to the control of infection. Moreover, NOD2 interacts with ATG16L1 which is crucial for all forms of autophagy. Of note, Crohn's disease may involve ATG16L1 polymorphisms as well as NOD2 polymorphisms [95]. Besides, genetic analyses have reported the role of ATG16L1, in addition to the pivotal role of another autophagy-related gene that is strongly associated with IBD which is IRGM gene [96-98]. Any mutation that occurs specifically in T300A gene or IRGM from the family of pp47 has been linked to an increased risk of Crohn's disease [99-101].

Several single nucleotide polymorphisms (SNPs) have been found in IBD patients in genes such as (TNFSF15) which encodes for TL1A (TNF-like molecule 1A) and is implicated in T cell response [32, 102, 103]. Other variant genes code different molecules which are involved in the differentiation and regulation of T cells such as IFNG, IL12B, IL2, IL21 [102]. It has been proven that the mutations in IL12B gene which normally encodes for the p40 subunit of IL-12 and IL-23 have an association with IBD and other immunological diseases. Also, variants in IL-10 have been involved in IBD [104]. Other genes play a major role in B cell response

and are associated with IBD also such as IRF5 and IL7R [102]. Moreover, there are some IBD susceptibility genes that are implicated in the endoplasmic reticulum (ER) stress pathway and are IBD-related such as XBP1 and ODL3 [105, 106].

Furthermore, a strong association between IBD (CD and UC) and IL23R has been revealed by researchers [66, 107]. Of note, IL23R is a gene which encodes the receptor of a pro-inflammatory cytokine IL-23 which is mainly involved in the production of Th17 cells. In addition, it has been shown that through modulating IL-23R recycling and cytokine production by macrophages, Arg381Gln, an uncommon variant at a highly conserved amino acid polymorphism, has a protective influence in individuals with Crohn's or ulcerative colitis [66, 108] .It should be noted that Th-17/IL23 pathway constitutes a crucial role in the pathogenesis of IBD, and that several gene loci are implicated in CD and UC such as IL23R, IL-12B, JAK2, and STAT3[109, 110]. New studies on human gene demonstrated the clinical advantages of IL-23 and anti-IL-23 R antibodies in the management of IBD. Besides, recent studies have shown that epigenetic modification which is the methylation of DNA and non-coding RNAs has an indispensable role in the initiation and course of the disease [111-114].

3.2 Smoking:

Research has proved that not only genetics play a major role in the pathogenesis of IBD, but also several environmental factors have a pivotal role in contributing to the disease, such as smoking, diet, social stress, medications, and geography [53]. It has been proven that smoking can act on the intracellular calcium found in T-cells [115], thus increasing the amount of CD4+ cells in the lungs which can further

release high amounts of inflammatory cytokines “interferon gamma”. Hence, moving to the intestinal area and causing inflammation [116]. In addition, smoking can act on the nicotinic acetylcholine receptors that are mainly located in the intestinal mucosal epithelial cells [115]. Further studies have reported that the chemicals found in the cigarette may modify the blood flow, increase the development of microvascular thrombosis, and alter the renewing of the intestinal mucus [117, 118].

In1982, the inverse association between ulcerative colitis and smoking was established, and it was shown that heavy smoking has a protective influence on the development of UC contributing to a lower relapse rate of the disease [119-121]. Studies have evidently proven that the protective effect of nicotine in UC is mainly due to the increased production of mucus, reduced production of nitric oxide and inflammatory cytokines, and to the enhancement of the intestinal barrier function [122]. However, it has been reported that smoking, in contrast to its effect on UC, raises the risk of CD and is linked to a higher rate of postoperative diseases [123]. Interestingly, nicotine causes an increased influx of neutrophils into the intestinal mucosa in the case of CD, hence worsening the case [122]. Additionally, it has been shown that it has a detrimental effect in impairing autophagy [124].

Of note, UC has been considered to be driven by a Th2 response, in contrast, CD has been associated with the Th1 response [125, 126]. Based on that, and knowing that nicotine is a major constituent in cigarettes, studies have shown that it has an indispensable role in inhibiting the function of Th2 cells which are associated with UC, without affecting the function of Th1 cells that are linked to

CD [127]. This explains why smoking increases the likelihood of CD without affecting the development of UC.

3.3 Stress:

It has been evidently confirmed that psychological stress is one of the important risk factors that may increase or initiate the likelihood of UC and CD [128, 129]. When stress is activated, various alterations in the hypothalamic-pituitary-adrenal (HPA) axis occur [130]. A study has demonstrated that people living in low stress conditions are less susceptible to developing IBD [131]. Knowing that stress is linked to the enteric nervous system (ENS) that regulates the motility of the gastrointestinal tract [132, 133], studies have proven that it may induce or reactivate the inflammation in the gut, thus worsening its clinical symptoms [134]. Camara RJ, et al., have reinforced the same concept by showing that anxiety and depression may strongly deteriorate those symptoms [135]. Interestingly, Goodhand et al. have reported in their study the decrease in the number of symptomatic relapses in IBD patients; fortifying the role of antidepressants in positively affecting the course of the disease [136]. Another study has contracted the result of the latter, reporting no effect of psychological interventions in IBD [137].

3.4 Nutrition:

Recent research has emphasized the critical role nutrition plays in altering different epigenetic pathways that may contribute to the development of IBD [138]. It has been observed that people in the western world are exposed to poor eating habits. This is mostly because of the increased variety, availability, affordability, and processing of food, [139, 140] which has led to dietary alterations, impairing the function and reducing the diversity of gut microbial composition as well as

degrading mucosal immunity [141-144]. A recent study has demonstrated that a typical Western diet given to the mice changed the gut microbiota's composition and colonic mucus' texture, exhibiting certain IBD symptoms. [144, 145].

In the Western countries, the intake of sugar-rich food has been predictable, showing that it has an association with Crohn's disease [146]. Moreover, it has been shown that food additives have contributed to promoting intestinal inflammation by disturbing the gut barrier function [139]. More in-depth studies have demonstrated that people consuming fruits and vegetables are less prone to the disease, which highlights their critical role in the protection against IBD [147]. Along this line, another study has reported that consumption of fruits and vegetables lead to a reduced risk of Crohn's disease [139]. Additionally, vitamin "D" has basically a role in the incidence of the disease [148-150].It has been pointed out that there is a positive association between vitamin "D" deficiency and IBD patients [151-153].In mice models, vitamin "D" has evidently caused a reduction in the severity of colitis, in addition to a reduction in the number of colorectal tumors in C57BL/6J and NOD2 deficient mice [154, 155].In other study, the deficiency of vitamin "D" has been prevalent in DSS- induced colitis mice models leading to a severe intestinal inflammation [156].However, other studies have reported that the link between vitamin "D" and IBD is still indefinite [157].

Furthermore, studies have shown a link between the start of IBD and a "High Fat Diet" (HFD). It has also been established that various polyunsaturated fatty acids (PUFA) have various impacts on the pathophysiology of the illness. For instance, those who consume omega-3 (PUFA) are less likely to develop IBD than people who consume omega-6 (PUFA). This is due to the fact that omega-3 has an

anti-inflammatory effect and omega-6 has a pro-inflammatory effect [158]. More research has indicated that a person's protein intake may be a risk factor for IBD. Animal-based proteins and plantbased proteins are the two main categories of proteins. Proteins derived from animals include more saturated fats than those derived from plants. According to Hou JK et al., only those who consume animal-based proteins, not those who consume plant-based proteins, have developed IBD [159]. A increased risk of IBD has also been demonstrated by Jantchou P et al for people who consume fish, which is mostly an animal-based protein. [160].

3.5 Medications:

Evidently, many drugs have been implicated in the development of IBD. They include non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, oral contraceptives, antibiotics, and hormonal replacement therapy [161-166]. For instance, the use of oral contraceptives in women is associated with a 1.5 folds increase of the risk of CD than non-exposed women [164]. Additionally, many studies have pointed out the relationship between the use of antibiotics and the risk of IBD [167]. However, a study has contradicted the above finding, reporting that the dose, duration, or frequency of aspirin use is not linked to the risk of UC or CD. It has confirmed that the use of frequent high doses of NSAIDs during a prolonged period of time has been prevalently associated with an increased risk of both diseases [163]. Moreover, a positive association between the exposure to antibiotics in childhood and the onset of IBD has been recognized. Shaw SY et al. have shown that antibiotic use is more common in pediatric IBD cases than in controls throughout the first year of life [168]. This might be due to the alterations that occur during the normal developmental process in the children’s gut microbiome [167].

In contrast, Gevers D et al. have shown in Asia the existence of a protective association between antibiotic use and the course of the disease [169].

3.6 Appendectomy:

Interestingly, appendectomy plays a role in affecting the incidence of both IBD diseases (UC and CD) in a paradoxical way. Well, it has been shown that appendectomy in patients with acute appendicitis and mesenteric lymphadenitis had a protective effect against UC but a deteriorating one for CD [170, 171]. It has been reported that the reason for appendectomy is a very important factor in shaping the outcome. In other words, if appendectomy is done due to a perforating appendix, then the patient would consequently have a higher risk for CD, but if other reasons are implicated, then the patient would have a lower incidence of the disease [171].

3.7 Ecological and Socioeconomic Factors:

Among the ecological and epidemiological factors, air pollution has been recognized as a risk factor for the development of UC and CD. Tan WC et al. have shown that there's a positive association between elevated level of air pollution and the increase of circulating polymorphonuclear leukocytes and plasma cytokines [172, 173]. It has evidently been shown that the increase of NO2 and SO2 levels in the air is mainly correlated to an increased risk of both diseases [174].

Moreover, the rise of IBD's incidence in developing countries has been associated with several socioeconomic changes influencing hygiene [175, 176] such as , absence of tap water, absence of hot water, consumption of contaminated food, lower birth rank, and large families having several children with crowded living conditions [177-179]. This is because excessive sanitation leads to a less exposure to environmental antigens, thus contributing to an impaired development

of intestinal a, resulting in inappropriate immune response later on when individuals are exposed to the same antigen for the next time.

4. Pathophysiology of IBD:

4.1 Microbial Factors:

The intestinal microbiome is thought to be made up of a large number of microbial species that coexist with the immune system to develop a strong symbiotic interaction. This connection is based on the provision of essential nutrients to the host, modulation of energy metabolism, provision of host defense, and immune system development. The formation of host-microbiome equilibrium is the result of this [180-188]. Short-chain fatty acids (SCFAs), which include butyrate, propionate, and acetate, are normally produced by the gut microbiota and play a crucial role in maintaining a healthy mucosa and releasing anti-inflammatory cytokines [189, 190]. In most healthy individuals, 99% of the bacteria are divided into four major groups: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, showing that Firmicutes and Bacteroidetes account for 90% of the total microbiota. It has been proven that the majority of bacterial colonies are found in the colon [191-194]. Moreover, it was found that strains of Lactobacilli that are members of the phylum Firmicutes have been essential in preventing the formation of numerous Gram-negative pathogenic bacteria and preserving the mucosa's good health.

Pathogen-associated molecular patterns (PAMPs), which are present in epithelial and immunological cells, are generally identified by pattern recognition receptors (PRRs involving NOD-like, Toll-like, C-type lectin receptors, and RIG-like receptors) [195197]. This stimulates the innate immune system, activates NF-

B and inflammasome, and produces proinflammatory cytokines to maintain intestinal homeostasis [198-201]. Under normal circumstances, a number of mechanisms, including those involving

immunological and epithelial cell molecules like RegIII, IgA, and defensins, control the microbiota, which controls the activation of the immune response and favors the production of specific T cell subsets [202]. Unless there is a disturbance brought on by a disease, exposure to antibiotics, or dietary change, intestinal homeostasis is preserved. [203, 204]. Gut dysbiosis results from an imbalance in the diversity, composition, and function of the microorganisms in the gut, which results in improper immunological activation [205-207]. It has been demonstrated that intestinal dysbiosis is a crucial contributor to the pathogenicity of IBD, strengthening the theory that IBD may be caused by intestinal immune system dysregulation, which in turn leads to an immunological response that is pathogenic [208, 209]. Since butyrate is a SCFA that is necessary for maintaining intestinal homeostasis, studies have demonstrated that a decrease in species that produce butyrate is observed in IBD patients [210-212]. 132 IBD patients have been enrolled in a research to evaluate their microbial activity as the disease develops. During the progression of IBD, it was found that there was a clear gut dysbiosis, the microbial composition had changed, and the microbial transcription was disrupted [213]. Moreover, intestinal microbiota has also been shown to be crucial in the majority of animal models of colitis [201]. Further research has demonstrated how dysbiosis causes alterations in the gut's microbial diversity in both UC and CD [214].

Several variations in bacterial diversity, abundance, and composition have been seen in patients with active disease when compared to healthy people [215]. IBD

patients have been shown to have an increase in mucolytic and pathogenic bacteria, which contributes to the breakdown of the mucosal barrier [214, 216-218]. Other research has shown that these patients exhibit a decrease in Firmicutes and an increase in Proteobacteria, including Bilophila, Enterobacteriaceae, and particular members of the Bacteroidetes, demonstrating the prevalence of temporal instability in the dominating taxa in UC and Crohn's diseases [169, 207, 210, 219-223]. Other studies have also demonstrated that CD patients exhibit decreased levels of Clostridium cluster XIVa, Bifidobacterium adolescentis, Dialister invisus, and Faecalibacterium prausnitzii [224-226]. Inflamed and non-inflamed tissues within the same human have recently been found to differ in microbial diversity. For instance, compared to non-inflamed areas, inflamed regions in CD patients have shown a lower total bacterial diversity [227]. Additionally, compared to healthy people, UC patients have a higher overall diversity of invasive bacteria found in the colonic mucus layer, including "Fusobacteria" [216, 228]. Furthermore, rectal enema using human isolates of Fusobacterium varium clearly demonstrated colonic mucosal erosion in mice. [229].

4.2 Immunological Factors:

It has been demonstrated that the healthy intestinal mucosa's innate and adaptive immune system play a significant role in regulating the level of low-grade inflammation [230]. The innate immune system is recognized as the body's first line of defense against foreign antigens, offering protection in the minutes to hours immediately following the onset of an infection. Natural killer cells (NKTs), macrophages, neutrophils, endothelial cells, and mucosal epithelial cells make up the majority of it. It is distinguished by a nonspecific response in which invading microbes are swallowed and digested by macrophages, allowing the adaptive

immune system to finish its job of providing protection. However, the adaptive immune system, which consists of humoral and cellular immunological responses involving the activation and proliferation of both B- and T-lymphocytes, is renowned for its selectivity against invaders. Unlike the innate immune system, the adaptive immune system requires days, not hours. The adaptive immune system, which has an advantage in developing memory B- and T-cells, takes days rather than hours to be activated in contrast to the innate immune system. Notably, the adaptive system's unique function is the essential foundation for the total eradication of infectious germs. Toll-like receptors (TLRs) have been proven to be essential components of the innate system where they identify and bind a wide range of viral, fungal, and bacterial antigens invading the host. The pathophysiology of many inflammatory conditions, including IBD, can be caused by the activation of defective TLR signaling, which can negatively affect the host and cause an unfavorable immune response. According to research on IBD, patients' macrophages and intestinal epithelial cells express TLR-2 and TLR-4 more than normal. TLRs are crucial in the beginning of the inflammatory cascade, since it has been shown that the decrease of TLR-4 expression in an animal model of IBD provided protection against colitis [231].

The immune system, in addition to the non-immune system which is mostly made up of endothelial cells, nerves, mesenchymal, and epithelial cells, both play a role in the pathogenesis of IBD, as was previously discussed [3, 59, 60, 209, 231]. Immune responses come in two varieties: humoral and cell-mediated. While effector T cells, macrophages, and neutrophils are stimulated by the cell-mediated immune response, B-lymphocytes are activated by the humoral immune response

and eventually secrete antibodies. It is understood that antigen-presenting cells such dendritic cells, macrophages, and intestinal epithelial cells attach to the foreign antigen, digest it, and then deliver it on their surface to CD4+ T-helper cells. T-lymphocytes are activated and developed into Th-1, Th-2, Th3, or T-regulatory-1 (Treg) lymphocytes in the lamina propria (intestinal epithelial cells and dendritic cells) or in mesenteric lymph nodes (dendritic cells). It is clear that Th-1 cells influence cell-mediated immunity by producing IL-2 and interferon gamma. Th-2 cells, on the other hand, promote the humoral immunity by increasing the synthesis of antibodies and the secretion of IL-4, IL-5, IL-10, and other cytokines. It has been demonstrated that IFN enhances the activation of macrophages, which in turn encourages the production of other cytokines including IL-12 and tumor necrosis factor [232]. IFN is produced by Th-1 cells (TNF). IL-12 stimulates T cells to differentiate into Th-1 even more. However, T-cell IL-4 induces Th-2 differentiation. In addition, transforming growth factor is produced by Th-3 cells, IL-10 by T-reg1 cells and Th-2 cells (TGF). It's interesting to note that research has shown the importance of IL-10 and TGF in reducing inflammation. Additionally, research has shown the importance of Treg-1 cells, Th-3, CD4+ CD25+ T-cells, and B-cells in the immunoregulation mechanism, particularly in bolstering the relationship between the innate and adaptive immune systems. Nonimmune cells also have a significant role in modifying an immune response, in addition to immune cells. They involve the elements of the intestinal extracellular matrix, such as fibroblasts, intestinal epithelial cells, granulocytes, adhesion molecules, and matrix metalloproteinases. A significant effect of this intercalating network on inflammatory regions results in a reduced inflammatory state or possibly full

healing [232]. In addition, neurons are well known for their crucial function in controlling immunological responses; they stimulate mast cell production of histamine and substance P, which increases intestinal vascular permeability. The production of IL-11 and other cytokines by fibroblasts, on the other hand, is known to occur. Now, with regard to adhesion molecules, it has been demonstrated that the selectin and integrin families, as well as the immunoglobulin (Ig) supergene family, including (intercellular adhesion molecule ICAM), are all expressed on the surface of leukocytes or endothelial cells, drawing them into the mucosa and causing their adherence in addition to their transendothelial emigration [233, 234].

It has been shown that IL-6 has a significant role in the etiology of the disease with regard to cytokines released during episodes of inflammation, and more specifically IBD [235, 236]. It has been demonstrated that IL-6 plays a role in the etiology of both colorectal cancer and UC [237]. Furthermore, it is clear that the treatment of monoclonal antibodies in mouse models of colitis induced by DSS has helped to neutralize and lower levels of IL-6 and TNF-α. This demonstrates that during inflammation, IL-6 performs a pro-inflammatory effect. However, it has been noted that IL-6's pleotropic effects are mostly determined by the stage of inflammation as well as the target cell. For instance, when inflammation begins, IL-6 prompts mesenchymal and epithelial cells to entice macrophages and polymorphonuclear leukocytes (PMNs) and begin the process of wound healing [238]. IL-6 inhibition has been demonstrated to suppress intestinal inflammation caused by Th cells and to inhibit T-cell proliferation [239, 240]. The signaling of IL-6 on intestinal epithelial cells (IECs) has been shown to activate the YAP/notch signaling pathway, which promotes the differentiation of absorptive epithelium and

further activates the repair pathways [241]. Furthermore, IL-6 increases STAT3 signaling in IECs, supporting barrier function and mucus secretion, according to a study [242].

In addition, other cytokines other than IL-6 have been linked to the pathophysiology of IBD, including members of the IL-1 family of cytokines (IL-1 and IL-18). Inflammasome component caspase-1 was deleted, which reduced the expression of IL-1b and IL-18. This reduced the severity of DSS-induced colitis in mouse models [243]. IL8 has been known as a chemoattractant that targets neutrophils which migrate from peripheral blood into inflamed areas. A study has reported an increase in IL-8 expression in UC patients compared to normal [244].

Regarding IL-9, it has been shown that Th9/IL-9 pathway plays a pro-inflammatory role in IBD, and in UC specifically. Its presence mainly aggravates the course of intestinal inflammation, and the administration of IL-9 antibody has been shown to ameliorate oxazolone-induced murine colitis [245].

In contrast, the presence of IL-10 has a protective role against inflammation. A study has reported that T-cells which lack IL-10 receptor are more prone to developing colitis [246]. Similarly, the absence of IL-21R aggravates the case of DSS-induced colitis [247]. A study has demonstrated that UC patients have increased expression of IL-33 and its receptor ST2 [248]. Regarding IL-23 signaling, it has been shown that IL-23 in activated Th17 cells induces JAK2 and STAT3 signaling contributing to the production of IL-17 and IL-22 [249].

Interestingly, the overexpression of STAT3 can induce the differentiation and proliferation of Th17 cells. Thus, the absence of STAT3 can absolutely prevent the differentiation of naïve T-cells into Th17 cells [250]. Well, in a DSS model of

colitis, the contrasting roles of IL-17A and IL-17F have been demonstrated. An excessive inflammation and deterioration of intestinal epithelium has been reported after the administration of IL-17A antibody [251, 252]. Whereas, lacking IL-17F in DSS-induced models of colitis has consequently contributed to the improvement of intestinal inflammation [253].

It has been proven that Toll-like receptors (TLRs) have specific sensing mechanisms in the gut microbiome which normally control the immune responses and maintain intestinal homeostasis [254]. Normally, when TLR2 is stimulated, it induces the association with the adaptor protein myeloid differentiation primary response protein 88 (MyD88) and recruits further proteins leading finally to the activation of NF-KB [17]. However, studies have shown that an alteration in TLR2/6 signaling leads to colitis [255, 256]. Shmuel-Galia L, et al have proven that the inhibition of TLR2 in this pathway has led consequently to the amelioration of colitis in DSS-treated mice [257]. Interestingly, the differentiation of more pathogenic Th1 and Th17 cells is caused by the upregulation of TLR6 expression and thus leading to colitis in mice [258].

NOD2 (nucleotide binding oligomerization domain 2) pathway has been considered to be one of the important pathways involved in IBD. It has been reported that NOD2 gene was strongly associated with IBD [62, 81, 259, 260]. Normally, NOD2 senses muramyl dipeptide (MDP) and activates NF-KB via receptor- interacting serine/threonine-protein kinase 2 (RIPK2) which associates with the inflammasomes to induce the secretion of IL-1b [261, 262]. It has been demonstrated that NOD2 deficiency in mice contributed to modified TLR signaling and production of high levels of proinflammatory cytokines by macrophages leading to inactivation of NF-KB and subsequently promoting injury [263].

IL-22/IL-22R pathway has been implicated in the pathogenesis of IBD. The stimulation of mononuclear phagocytes (MNPs) by commensal microbiota has triggered the secretion of several interleukins such as IL-1b, IL-6, and IL-23 leading to the activation of innate lymphoid cells (ILC3s) inducing IL-22 production [264]. It has been shown that STAT3 and MAPK pathways are both activated as IL-22 binds to its receptor, contributing to the production of IL-10, mucus, and antimicrobial peptides (AMPs). In addition, IL-22 has been shown to have an anti-apoptotic role, thus enhancing the proliferation of the damaged intestinal epithelium via the increase of claudin-2 expression [265]. In this context, a study has proven that colitis is exacerbated in mice models after the blockade of IL-22/IL-22R pathway; this shows that IL-22 pathway has a protective role against the development of colitis [265, 266].

Type1 Interferon (IFN-1) mainly promotes intestinal epithelial integrity through the activation of STAT1 and STAT2 signaling pathways [267]. However, mutations in type1 Interferon receptor gene (IFNAR1) occur, contributing to IBD pathogenesis [268]. In addition, it has been demonstrated that tumor necrosis factor-α (TNF-α) plays a vital role in IBD through triggering the upregulation of IL-1b, IL-33, and IL-6 levels [269, 270]. In contrast, transforming growth factor- ß (TGF-ß) has been shown to suppress the development of T-cell mediated colitis [271].

Hence, the strong connections during inflammatory episodes between immune cells which produce chemokines and cytokines and non-immune cells such as adhesion molecules leads to an enhanced production of cytokines, neuropeptides, growth factors, and antibodies. In addition to oxygen and nitrogen reactive species

which all participate in the pathophysiology of inflammatory diseases such as IBD [272].

4.3 Oxidative Stress in IBD:

It is well known that the survival of a mammalian cell depends on oxygen metabolism which usually leads to the production of reactive oxygen species (ROS). ROS are either free radicals or nonradical molecules. Free radicals mainly include superoxide (O_2^-), hydroxyl radicals (HO^-), alcoxyl (RO^-), peroxyl (RO_2^-), and hydroperoxyl (HO_2^-), in addition to lipid hydroperoxides. While neutral or nonradical compounds involve singlet oxygen (O_2), ozone (O_3), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), and chloramines (RNHCl) [273]. Since ROS are characterized by their unstable conditions with unpaired electrons, they are considered to be highly reactive, thus causing a series of intracellular damages of nucleic acids, lipids, proteins, and carbohydrates [274, 275]. Mainly, several intracellular organelles are responsible for the generation and production of endogenous ROS. These organelles involve endoplasmic reticulum, mitochondria, peroxisomes, nucleus, cytosol and extracellular matrix, in addition to the mitochondrial electron transport chain which mainly generates a large proportion of ROS [276, 277]. Interestingly, the mitochondria are central organelles considered as the powerhouses of the cell; being responsible for producing and metabolizing energy. It is known that the production of excessive ROS contributes consequently to a decreased level of ATP generation, leading further to the inhibition of the intracellular electron transport chain, and finally to the DNA damage inside the mitochondria. Such process can disrupt the mitochondrial homeostasis if kept in progress, leading to apoptosis [278, 279]. Many studies have reported that the

mitochondria produce ROS when an alteration in the physical state of its membrane, such as fluidity, occurs [280].

Normally, an oxygen plays a vital role in aerobic respiration where it accepts the last electron at the end of the respiratory chain, and subsequently reduces itself to water (H_2O) [281, 282]. Interestingly, a small portion of the transported electrons react directly with oxygen before reaching the final part of the respiratory chain (cytochrome oxidase), leading to the formation of superoxide (O_2^-) [283]. It has been reported that 1%-2% of the electrons traveling through the mitochondrial chain leak out, react with free oxygen, and produce superoxide and hydrogen peroxide (H_2O_2) [284, 285]. The latter serves as a precursor to the formation of hydroxyl radical (OH^-) when iron ions are present in excess in the medium of the reaction [286-291].

Furthermore, superoxides act as precursors to many ROS, yet they don't acquire the highest reactivity of the species [292]. They are produced endogenously, and are mainly generated through oxidase's action in postischemic tissues [293, 294]. They are generated from a plethora of sources; the cytochrome P450 system, redox cycling substrates, soluble oxidases in phagocytic cells, and arachidonic acid metabolizing enzymes; cyclooxygenases (COXs) and lipoxygenases (LOXs) [281, 282, 295-301]. Also, NO synthase is an enzyme that leads to the generation of another free radical (NO^-) from Larginine, and thus producing superoxide when its cofactor "tetrahydrobiopterin" is oxidized into "dihydrobiopterin" [302].

Moreover, it has been shown that ROS has a vital role in activating calcium channels in humans, specifically in their amnion cells, thus leading to an increase

in the intracellular calcium concentration [303]. Similarly, Brott T. et al. showed that an increase in the intracellular calcium concentration was caused by an inhibition of the calcium-ATPase which was consequently caused by ROS [304].

It should be noted that the gastrointestinal tract constitutes a major source of ROS production. During inflammation, tight junctions in the intestinal barrier are destructed, and the permeability of the gastrointestinal epithelial cells is subsequently increased, leading to the production of several proinflammatory mediators (such as cytokines and ROS) [305]. When the level of oxidative stress and nitrosative stress increases due to ROS and RNS, a pathogenic cascade consequently starts, thus triggering an inflammatory response in the intestines and causing IBD [282, 306]. It should be noted that a vast amount of neutrophils and macrophages is triggered during episodes of inflammation in IBD; called "intestinal mucosal infiltration" [307].

It has been shown that the level of RONS is highly increased during the episodes of chronic intestinal inflammation, specifically in the early stages of the disease (IBD), and such increase assists in the development and progression of both ulcerative colitis [275] and Crohn's disease [274]. However, several studies have reported the absence of a significant association between the severity of the disease (IBD) and oxidative stress [308-311]. Ingraham LM et al. in their study have reported how specific proinflammatory mediators such as LTB4 and platelet activating factor (PAF) mediate the activation of phagocytes during the episodes of inflammation, thus leading to the generation of a huge amount of ROS metabolites [312]. Additionally, Sharon P et al. have emphasized on what Ingraham et al. have

proven, and have continued to show that the mucosal samples from IBD patients do possess high levels of both LTB4 and PAF [313].

Regarding PDGF-BB factor, it has been shown that PDGF expression is upregulated during active episodes of IBD [314]. In addition, iNOS expression and nitric oxide levels have been significantly associated with the appearance of IBD symptoms in rat models such as diarrhea [315]. Other studies have shed the lights on the severe damage that may occur in the mucosal and submucosal intestinal cells due to the action of these compounds [316]. Furthermore, an increase in myeloperoxidase (MPO) expression has been strongly implicated and demonstrated in inflamed colonic biopsies from UC patients, showing a strong correlation with disease progression [317, 318].

Accumulating evidence has shown that in IBD, there's an imbalance that occurs between ROS and antioxidant activity; either an increase in the production of ROS or a decrease in the antioxidant activity [283]. Such imbalance is known as oxidative stress that is implicated in IBD and many other diseases such as diabetes, cancer, atherosclerosis, and cardiovascular [319].

Well, the production of ROS has been always controlled by the production of an antioxidant system that involves a plethora of enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPXs), and their antioxidant substrates (glutathione and α-tocopherol) [320]. In addition to other antioxidant compounds such as ß-carotene, vitamin C, bilirubin, zinc, selenium, copper, and urate [321].

One of the well-known antioxidants is “Superoxide dismutase” (SOD) which has been expressed in three different isomer forms (SOD1, SOD2, and SOD3) in IBD patients. It has been shown that its activity is correlated with the course of the disease [322]. The expression of SOD2 has shown a significant increase in IBD, while a downregulation of SOD3 has been detected especially in intestinal epithelial cells (IECs). Well, regarding SOD1, no effect has been reported [323]. Moreover, several studies have demonstrated contradictory results regarding SOD’s expression in IBD animal models of colitis. For instance, in studies on UC induced by intrarectal acetic acid, an increased SOD level has been detected [324]. Whereas, other studies on UC induced by TNBS have reported a reduced SOD activity [325-327]. This implies that the level of oxidative stress has increased, leading further to the oxidation of proteins and membranes’ lipid peroxidation, therefore damaging cells [328].

“Catalase enzyme” (CAT) is found in peroxisomes and is chemically responsible for reducing hydrogen peroxide into water and oxygen [329]. Clinical studies have proven that CD and colorectal cancer patients have both downregulated CAT levels [330, 331]. Also, a decreased expression of CAT in genetically modified mice has been linked to a decreased occurrence of colitis and colon cancer too [332, 333].

“Glutathione peroxidase” (GPX) is another antioxidant which has mainly three different isoforms. Well, the polymorphisms occurring in GPX1 and GPX2 levels have resulted in intestinal inflammation and oxidative stress, in addition to the appearance of IBD symptoms in mice models [334]. Other studies have demonstrated the significant association between increased GPX2 levels and colitis

[335, 336]. In mice models of colitis treated with DSS, the plasma glutathione peroxidase (E-GPx) has been increased by more than 60% compared to the control, showing that GPX is implicated in the pathogenesis of the disease [337].

One of the intracellular non-enzymatic antioxidants is glutathione (GSH). A sharp increase in its expression has been shown during episodes of inflammation [338], whereas in animal models of colitis, a reduced expression of GSH has been detected [325, 339, 340]. A study has demonstrated a low level of GSH in DSS-induced models of colitis, and that antioxidants play a main role in restoring its level to normal [334, 340, 341]. Furthermore, "Mannosylerythritol Lipid" (MEL) is evidently known for its strong antioxidant role in several inflammatory diseases involving IBD. Additionally, vitamins, transferrin, lactoferrin, albumin, uric acid, and ascorbic acid have been explicitly shown to play a pivotal role as plasma antioxidants in IBD [282, 320]. CD patients have been detected for their low blood and mucosal levels of vitamins A, C, E, and □-carotene [342346]. It is worth saying that minerals such as zinc, iron, copper, and manganese are necessary for antioxidant enzymes. For instance, CAT's activity requires irons and SOD's activity requires zinc, copper, or manganese [347]. There are also extracellular antioxidants that are usually found in fruits and vegetables such as polyphenols. One of them is "Flavonoid" which inhibits the activity of several ROS enzymes including COX, LOX, NOX and many others [348, 349].

Many signaling pathways have been implicated in IBD pathogenesis and are related to ROS generation. One of them is "NF-κB pathway". Well, after microbial invasion, TLRs and TNFs are both activated. Subsequently, the intestinal epithelial

cells (IECs) recognize this activation, thus leading to the events of NF-κB signaling. TNF-α causes the production of NOX1 and NADPH oxidase organizer 1 (NOXO1) in the colon cells leading further to the generation of superoxide [350]. Beside ROS production and NOX activation, LC8 peptide is released and IκBα is phosphorylated [351]. The activation of this pathway leads to the production of several pro-inflammatory cytokines such as IL-6, IL-16, IL-8, and TNF-α, in addition to the transcription of p53-upregulated modulator of apoptosis (PUMA) which further contributes to the apoptosis of epithelial cells and UC development [352]. Furthermore, NF-κB activation leads to the upregulation of myosin light chain kinase (MYLK) which degrades myosin in the intestinal barrier, as well as it leads to the production of metalloproteinases (MMPs), COX-2 and iNOS enzymes [352, 353]. Studies have evidently revealed the association between oxidative stress and activation of NF-κB pathway in IBD [351]. More in depth studies have shown that phenyl-N-tert-butylnitrone, an antioxidant drug, ameliorates inflammation in DSS murine models, and this is actually due NF-κB inhibition [354].

Moreover, Nuclear factor -Erythroid 2-Related factor2 signaling pathway plays a critical role in the antioxidant mechanism in IBD, for its responsible for maintaining intestinal mucosal homeostasis by inhibiting the generation of excessive ROS. It has been shown that *Nrf2*-KO mice revealed a reduced expression of antioxidant enzymes with an increased expression of IL-1β, IL-6, IL-8, iNOS, and COX-2. Another study has validated the presence of more severe symptoms of IBD in DSS-induced *Nrf2*-KO mice model of colitis compared to the wild type mice, showing that the knock-out of *Nrf2* exacerbates the course of the disease [355, 356]. Interestingly, this pathway is related to phosphatidylinositol 3

kinase/protein kinase B (PI3K/Akt) pathway through *immediate early response-3* (*IER3*) gene. It has been demonstrated that the absence of (*IER3*) in colitis model induces the activation of Akt and Nrf2 contributing to the reduction of ROS generation and apoptosis [357]. This explains why the activation of (*IER3*) in IBD leads to a decreased *Nrf2* expression. However, in normal cases, *Nrf2* increases the level of antioxidants GSH, thus inhibiting protein kinase "C" and reducing the activation of NOX. On the other hand, the absence of *Nrf2* in *Nrf2*-KO mice leads to the activation of NOX and protein kinase "C", thus causing a decreased level of antioxidants [358].

5. Animal Models of IBD:

The multifactorial nature of IBD has emphasized the need to develop a vast number of animal models that served as unique tools for a better understanding of the disease pathogenesis and the underlying mechanisms involved in its initiation and progression. Researchers attempted to induce the disease (in its acute and chronic forms) in those models in several ways, thus allowing them to express various defects mimicking its different aspects [69]. Such models have provided pivotal insights into histopathological and morphological modifications occurring in the intestinal tract during the course of the disease [359]. However, it has been shown that none of these models has provided a sufficient clinical and histopathological representation of IBD, yet they have elucidated a detailed understanding of its pathogenesis [360, 361]. Importantly, researches focused on those models in research to use them for preclinical studies of drug development [360]. These models have been categorized into chemically induced colitis, spontaneous colitis, adoptive transfer, and genetically modified models [246, 359,

362-364]. The chemically induced models were the first to be used by Kirsner and Elchlepp in 1957 when they induced colitis in a rabbit model by irritating its rectum with a diluted formalin solution, and then localizing its crystalline egg albumin within its colon [365]. After several years, chemically induced murine models of colitis were developed; the dextran sulfate sodium (DSS) and the 2,4,6-trinitrobenzene sulfonic acid (TNBS) models. In the following years, more than 60 different types of acute and chronic models of intestinal inflammation were generated [366]. In 1981, spontaneous colitis was induced in the cotton –topped tamarind animal model which used to live in Colombia. Nine years later, Dr. Powrie and other researchers induced experimental colitis in immunodeficient mice using an adoptive Tcell transfer system; they transferred lymphocytes in lymphopenic mice [367]. Interestingly, this model system has significantly led to a deeper understanding of the vital role of regulatory T-cells and the regulation of the mucosal immunity [368, 369]. Cell-transfer models include CD45RB-^{high}, CD62L-^{high}, and Hsp60 $CD8^+$ T cells [370].

In 1990, colitis was developed in genetically engineered modified rats that carry (HLAB27) gene, a specific human gene [371]. In 1993, three different types of knockout (KO) mice which are interleukin-2 (IL-2) KO, interleukin-10 (IL-10) KO, and T-cell receptor (TCR) KO mice also developed spontaneous colitis [372]. Additionally, it was developed in transgenic (Tg) models where a specific gene has to be selected, overexpressed, and introduced into such models leading to intestinal inflammation. More than 40 different types of genetically engineered mouse strains in addition to other strains possessing congenital gene mutations develop spontaneous colitis and/or ileitis. Genetically manipulated models include STAT3

KO, TGFβ-1 KO, Caspase-8 KO, XBP-1 KO, Keratin-8 KO, WASP KO, NEMO KO, STAT4 Tg, IL-7 Tg, Gp39 Tg, and many others [370]. Moreover, it has experimentally shown that the mating of two different inbred strains of mice having different genetic backgrounds caused spontaneous models of intestinal inflammation, and such models represent congenic models of IBD [366]. Congenic models are characterized by their complexity and similarity to human immunopathogenesis, thus they are poorly understood by researchers. In other words, a congenic model is the most relevant to human conditions and diseases [373]. They include C3H/HeJBir, SAMP1/Yit, and cotton-top tamarin models [370]. However, most of the researchers tended to use the chemically induced models instead due to the direct procedures they require, in addition to the rapid onset of inflammation they develop consequently [362, 374].

As compared to genetically modified models, chemically induced models are better in avoiding different developmental abnormalities that can be the result of a certain genetic defect that takes place in genetically engineered models. Additionally, they have an advantage over the cell-transfer system which requires the use of immunodeficient mice to be successful. In contrast, chemical induction is applied in immunocompetent mice which is much easier in performing experiments. Furthermore, researchers have to carefully select the model taking into consideration the standardized experimental design being used because such factors are very critical in affecting the extent of the disease development. It should be noted that such models are easily used in the field of IBD research. Specifically, chemically induced models serve as pivotal tools in mimicking some of the histopathological and pathophysiological aspects of the disease, thus paving the

way to important therapeutic interventions and strategies to treat IBD. Although these models have provided great insights into the disease pathogenesis, still none of them have fully elucidated its complexity and heterogeneity in humans [375]. There are several chemical induced models including dextran sulfate sodium (DSS), 2,4,6-trinitrobenzene sulfonic acid (TNBS), dinitrobenzene sulfonic acid (DNBS), acetic acid, oxazolone, carrageenan, peptidoglycan polysaccharide, immune complex, indomethacin, and cyclosporine A. Most of the researchers have commonly used the DSS and TNBS as models of colitis [370].

6. DSS Model of Colitis:

Knowing that DSS model is one of the most commonly used chemically induced models of IBD, we induced in our research project "IBD" in mice using DSS, and this is due to several reasons. First of all, DSS is mainly a water-soluble polysaccharide that is made up of dextran and sulfated anhydro-glucose unit [376], carrying a highly negative charge due to its sulfated groups. Its molecular weight ranges from 5 to 1400 kDa. It has been shown that supplementing the drinking water of murine models with DSS (40-50 kDa) has led subsequently to murine colitis [377]. Additionally, Kitajima et al. reported that a mild colitis is caused by a low molecular weight DSS (5 kDa) while a high molecular weight (500 KDa) doesn't contribute to colonic injury [378]. Due to unknown reasons, the huge pathology of the disease induced by DSS has been limited to the large intestine, in specific the distal colon [379]. Laroui et al. have suggested that DSS-induced colitis is associated with the formation of nano-lipocomplexes with medium-chainlengthy fatty acids (MCFAs) in the colon [380]. It has been shown that the oral administration of DSS via drinking water in mice had disastrous effects on their gut

epithelial cells and the integrity of their intestinal mucosal barrier [381]. It mainly contributes to epithelial injury in the gut; exposing the mucosal and submucosal immune cells to foreign antigens and promoting a severe inflammatory response [375].

Consequently, an upregulation in the levels of nitric oxide, inducible nitric oxide synthase (iNOS), and chemokines has been noticed [382, 383]. Yan et al. have evidently reported the increase in the levels of proinflammatory cytokines after the administration of DSS also [384]. The assessment of different inflammatory mediators has played an important role in understanding the pathology of DSS-induced colitis; it included TNF-α, IL-1ß, IL-6, IL-17, IL-10, TGF-ß, mucin, TLR2/4 gene expression, and MPO activity [359, 362]. In addition, several changes in the expression of the tight junctions have been reported one day post treatment with DSS [385].

Several researchers have used the DSS model of murine colitis to study the association between colonic inflammation and Gall-R expression. The results of their experiment have evidently demonstrated an induction of a progressive and severe colitis where its severity has been associated with the activation of NF- κB and a further increase in Gall-R expression. Researchers assumed that Gall-R expression might be a vital component in the excessive fluid secretions detected in IBD [386, 387]. In 2000, Soriano et al. used this model to study the involvement of several adhesion molecules (CAMs) considering their important role in the pathogenesis of human IBD (UC and CD), specifically in regulating the recruitment of leukocytes during inflammation. They have studied three different endothelial CAMs; the vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion

molecule 1 (ICAM-1), and mucosal addressing cell adhesion molecule 1 (Mad-CAM-1) which have been implicated in various acute and chronic inflammatory diseases [388].

Although the experimental protocol of DSS-induced colitis is easy, several factors affect its efficiency in animal models, involving concentration of DSS (usually 1% to 5%), molecular weight of DSS, duration and frequency of treatment (acute or chronic), strain(BALB/c and C3H/HeJ mice strains are more susceptible to the disease) and gender (males show increased susceptibility) of the animal model, and the microbial environment of the animals (if it is pathogenic or germ-free) [377, 389]. Interestingly, studies have highlighted the effect of enteric bacteria on acute colitis; it has been validated that the enteric bacteria suppress the development of the disease. Researchers kept mice in germfree conditions and after a while, they detected a massive intestinal bleeding in those mice as a result of DSS administration contributing to the development of lethal colitis [390].

Several acute histological modifications occur at the level of the colon after a short exposure to a relatively high dose of DSS. They include mucin depletion, epithelial erosion and necrosis contributing to the disappearance of epithelial cells. Araki et al. have proven that an acute phase of DSS is mainly characterized by the presence of a leaky epithelial barrier caused by a decreased proliferation of the epithelium in addition to an increased level of apoptosis [391]. It should be noted that during inflammation, neutrophils migrate to the inflamed site, thus forming cryptitis and crypt abscesses. Although those are considered to be important histological aspects of human IBD, they are rarely reported in DSS-induced colitis [386]. Several clinical symptoms have been observed when inducing acute colitis

such as bloody stools, weight loss, diarrhea, hunched back, and death [379]. However, a continuous administration of relatively low dose in a cyclic manner causes chronic lesions. A chronic phase of DSS-induced colitis is mainly characterized by an infiltration of mononuclear leukocytes, disarray in the crypt architecture; where the gap between the muscularis mucosa and crypt bases is widened, in addition to the formation of deep mucosal lymphocytosis and transmural inflammation [362].

Interestingly, DSS model is characterized by its simplicity, controllability, reproducibility, and rapidity in inducing inflammation [379]. Further, it has been used in screening potential therapeutic substances. Studies have elucidated that the induction of strong acute colitis by DSS in lymphopenic immunodeficient mice (SCID and Rag-/-) appeared to be linked with innate immune cells and not adaptive ones [392, 393]. Thus, acute models have offered insights only into innate immunity being more relevant in studies that address the physiology of acute flares, wound healing, and resolution of acute inflammation. Thus, providing limited information about the pathogenesis of IBD. Whereas, chronic models have been implicated in studying the adaptive immunity which seems to be an advantage in studying the complications of chronic diseases such as neoplasia and tissue fibrosis [375].

7. Syndecan-1 knock-out Model:

7.1 Definition of Syndecan-1 and its Role in Health:

Syndecan-1 or (CD138) is a member of the heparan sulfate (HS) proteoglygan family which is mainly expressed on the glycocalyx of epithelial cells, endothelial

cells, and plasma cells [394, 395]. It consists of three different domains; a long variable ectodomain, a single transmembrane domain, and a short cytoplasmic domain [394]. SDC-1 mainly modulates different proteolytic activities [396], and plays a crucial role in a plethora of biological processes involving cell proliferation, differentiation, and redifferentiation [397]. Also, it acts as a co-receptor for different tyrosine kinase receptors [398, 399]. For instance, it modulates and stimulates the activity of the complex of basic fibroblast growth factor (bFGF) and the FGF receptor, thus leading to keratinocyte proliferation and consequently to improved wound healing [400]. In addition to its role in integrin activity [401] and migration [402], it has been implicated in development, tissue repair, tumor progression, and inflammatory responses [395]. J. Angsana has proven that SDC-1 expression on macrophages enhances macrophage's motility in human and murine models, and is significantly associated with an anti-inflammatory M2 polarization [403]. SDC-1 has been involved in maintaining the function of the mucosa barrier. It seems to activate Stat3 leading to the restoration of the tight junctions' dysfunction in the cell, and thus maintaining the epithelial integrity [404]. The extracellular domain of SDC-1can be shed from the cell surface and released into extracellular fluids by the action of distinct metalloproteinases, cytokines, chemotactic factors, as well as oxidative stress [405].

7.2 Role of Syndecan-1 in Disease:

It has been shown that patients suffering from multiple myeloma, acute graft-versus host disease, diabetic nephropathy, acute coronary syndrome, cardiogenic shock, acute myocardial infarction, cardiac fibrosis, and severe sepsis, in addition to patients undergoing dialysis or major vascular surgery have increased levels of

soluble SDC-1 [405-415]. Additionally, it has been demonstrated that an elevated expression of SDC-1 has been mainly implicated in invasive growth and development of distinct tumor entities involving breast cancer and myeloma cancer [416-420]. Moreover, a clinical study has proven that colorectal carcinoma patients have significant high epithelial SDC-1 levels which is mainly associated with tumor size [421]. A further study highlighted the association between serum SDC-1 levels and Systemic Lupus Erythematosus (SLE), showing that this level is significantly upregulated in SLE patients with active disease compared to individuals with inactive one [422]. Knowing that SDC-1 plays a pivotal role in migration, it has been shown that the gene knockout and overexpression of SDC-1 lead both to a reduced migration in a wound healing process [400, 423]. H. SalminenMankonen et al. have proven that SDC-1 expression is upregulated in the knee joints of Dell mice during the early stages of articular cartilage degeneration, and that SDC-1 positive cells have been localized in chondrocytes that are close to degenerated areas [424]. G. Diab et al. have demonstrated the association between SDC-1 expression and Rhematoid Arthritis (RA) as well, showing that after a six-week of antirheumatic treatment, serum levels of SDC-1 have been reduced reflecting the reduction in SDC-1 shedding from glycocalyx [425]. Furthermore, it has been reported that SDC-1 deficiency leads to skin inflammation in experimentally induced psoriasisform dermatitis mouse models and other models leading to exaggerated airway hyperresponsiveness [426, 427].

7.3 Role of Syndecan-1 in IBD:

It has been shown that SDC-1 plays a crucial role in intestinal inflammation. Patients with chronic IBD have shown downregulated levels of SDC-1 in their colons [428, 429]. Specifically, patients with ulcerative colitis (UC) have increased SDC-1 shedding from their cell surface, leading to the activation of several inflammatory factors and higher circulation of neutrophils. Further results in that study have shown that cell-surface anchored SDC-1 inhibits the secretion of pro-inflammatory cytokines due to its suppressed ectodomain shedding, causing amelioration of intestinal inflammation and neutrophil transmigration [430].

It has been demonstrated that loss of SDC-1 contributes to an increase in epithelial permeability causing protein-losing enteropathy which is similar to the case of IBD where a defect in the intestinal epithelial barrier takes place [431]. It has been evidently revealed that inflammation disrupts the normal healing process in IBD, and this is maybe due to the loss of SDC-1, leading to a decreased rate of healing. Well, patients with ulcerated mucosa have actually shown a lack in SDC-1 expression and a decreased rate of tissue repair, consequently leading to a state of persistent chronic inflammation [431-433]. Another study has demonstrated the correlation between the release of TNF-α and IL-1ß by lamina propria mononuclear cells in active IBD and degree of mucosal inflammation [434]. The stimulation of HT29 and T84 colonic epithelial cells with TNF-α and IL-1ß leads to SDC-1 shedding, and thus downregulation of the epithelial SDC-1 expression [432]. Additionally, it has been reported that patients with CD have reduced SDC-1 levels which is mainly caused by upregulated TNF-α levels [432]. Importantly, several murine models of IBD have similarly shown reduced levels of SDC-1 [435-437].

For instance, Floer M. et al. have proven that syndecan-1- null mice have delayed and exaggerated recruitment of leukocytes, impaired mucosal repair, and worsening of DSS colitis symptoms with high lethality [438]. Another study has revealed that a DSS-induced model of colitis is characterized by an increased syndecan-1 shedding which leads to a reduced expression of anchored SDC-1 in the colonic mucosa. This explicitly proves the correlation between SDC-1 shedding process, decreased mucosal SDC-1 expression, and sustained inflammation [439].

8. Management of IBD:

The aim of having a treatment for IBD is mainly to maintain patients in remission, ameliorate the symptoms of the disease, reduce its severity, and prevent surgeries [440].

It is crucial to diagnose the symptoms of patients very well, and to specify the type of the disease before giving them a certain treatment. Specifically, treatment for IBD is mainly based on pharmaceutical drugs and self-care [441]. “Aminosalicylates” were the first to be used to treat IBD. It involves two different drugs, “Salazopyrin” which is the oldest one in this category, and “Mesalazine” which is the main aminosalicylate used in IBD management nowadays [442]. It has been proven that the effectiveness of aminosalicylates depends on the dose taken by patients [443]. Moreover, corticosteroids mainly “Hydrocortisone” and “Prednisolone” have been commonly used for treatment. Studies have shown that corticosteroids can be used either alone or in combination with “Mesalamine” which might be better and more effective [444].

Knowing that TNF-α is one of the proinflammatory factors produced during inflammation, several anti-TNF agents such as “Infliximab”, “Adalimumab”, and

"Golimumab" have been produced as classic IBD drugs. The combination of "Infliximab" and "Azathioprine" has been shown to be efficient in maintaining remission in both CD and UC patients [445-447]. However, "Vedolizumab", an α4β7 integrin blocker, is transcribed for those who don't respond to anti-TNF therapy [448, 449].

In addition, classical immunosuppressive drugs such as "Azathioprine", "Methotrexate", and "Cyclosporine-A" have been used in IBD therapy. "Azathioprine" has been shown to have a positive effect in both UC and CD cases while the effect of "Methotrexate" has been limited only to CD cases. Interestingly, "Cyclosporine-A" has efficacy in only UC cases [450-453]. It is very essential in patients who didn't respond to corticosteroid treatment, for that it might prevent them from surgery. However, after one year, it has been proven that most of the patients might require surgery [454]. "Cyclosporine-A" and "Methotrexate" have been shown to inhibit the production of proinflammatory cytokines and stimulate apoptosis [455-458].

9. Cyclosporine:

9.1 Definition of Cyclosporine "A" and its Role in Health:

Cyclosporine A is a large hydrophobic, cyclic, organic molecule that was extracted from the soil fungus *Tolypocladium inflatum Gams* in 1971 [459, 460]. After organ transplantation, it acts as an immunosuppressant to prevent rejection. In addition, it has a pivotal role in ameliorating chronic inflammatory diseases [461]. Cyclosporine downregulates the activation of T-lymphocyte by blocking the production of IL-2 through inhibition of the calcineurin pathway [462-464]. Its

mechanism of action starts when it binds to cyclophilin, an intracellular binding protein for CyA, forming a (CypA-CyA) complex. The formed complex binds to calcineurin, a protein phospahatase that has a vital role in T-lymphocyte activation, and prevents the dephosphorylation of the nuclear factor of activated T-lymphocytes (NFAT). Thus, the cytosolic component of NFAT can't enter the nucleus, and NFAT complex is not formed. Thereby, the production of IL-2 and its receptor are further inhibited [465]. Not only IL-2 is inhibited when the complex binds to calcineurin since calcineurin is a regulatory factor that leads to the transcription of several cytokine genes such as IL-3, IL-4, TNF-α, granulocyte-macrophage colonystimulating factor (GM-CSF), and interferon-γ (IFN-γ). Knowing that calcineurin controls the phosphorylation of nitric oxide synthase (NOS), and knowing that CyA prevents the calcineurin pathway, it has been shown that CyA reduces the production of nitric oxide, thus affording an anti-inflammatory activity [466].

In addition, it has been proven that CyA inhibits the activation of c-Jun N-terminal kinase (JNK) and p38 signaling pathways [456]. Interestingly, it contributes to the apoptosis of CD4+ T-lymphocytes [467] and prevents the activation of antigen presenting cells, specifically B-lymphocytes [464]. Evident studies have shown that it plays a critical role in impeding granulocyte infiltration, thus leading to an increased vascular permeability [466]. Concerning that, CyA inhibits NF-KB and reduces the expression of cellular adhesion molecules, contributing further to a decreased adhesion contact, transendothelial migration, and neutrophils' infiltration [468]. Moreover, it has a pivotal role in the blockade

of chemotactic migration and inhibition of superoxide and IL-8 production in a dose-dependent manner [459].

9.2 Role of Cyclosporine "A" in IBD:

It has been known that cyclosporine acts as a treatment for IBD patients since the mid-1980s and has been used as a substituent for corticosteroid therapy in UC patients [444]. Additionally, it has been reported that cyclosporine therapy plays a pivotal role in fistula closure in 44% of CD patients [469]. It is known that cyclophilin is an endogenous protein secreted by activating macrophages and has a pro-inflammatory effect. When CyA binds to cyclophilin, it neutralizes its chemotactic properties, thus inhibiting intestinal inflammation [459]. Evident studies have reported an increase in the expression of several cytokines such as TNF-α, IL-4, and IL-17 in IBD patients and IL-10 in UC patients [269, 470, 471]. However, a significant decrease in their expression has been detected in IBD groups treated with CyA [455]. It has been demonstrated that cyclosporine A is an effective treatment in reducing colitis [472]. Sumit Sharma et al. have proven that TNBS induced colitis in New Zealand rabbits is ameliorated after using a targeted delivery system of cyclosporine, and that the expressions of TNF-α, IL-6, IL10 are downregulated in treated animals [473]. Additionally, in a DSS-induced model of colitis, cyclosporine has ameliorated body weight loss, epithelial apoptosis, and colonic mucosal destruction [474]. Similarly, it has particularly prevented the presence of ulcerations and lymphocytes' infiltration in treated animals. In addition, it has avoided colon shortening which is usually detected as an inflammatory marker, and has decreased the production of pro-inflammatory cytokines [475]. In another study, Holger Sann et al. have shown that systemic and colonic anti-DSS

effects are produced after the oral treatment of cyclosporine A in a dose-dependent manner [475]. However, serious adverse effects have been evaluated in IBD patients taking intravenous CyA (4 mg/kg/day) followed by oral CyA (8 mg/kg/day). They involve renal insufficiency, hypertension, hypomagnesemia, paresthesias, seizures, anaphylaxis, infections, and death [476].

10 Cyclophilin:

10.1 Definition of Cyclophilin:

Cyclophilins (CyPs) are ubiquitous proteins which are well conserved and present in both prokaryotes and eukaryotes [477]. The peptide bonds of CyPs are isomerized from trans form to cis form at specific proline residues, and this isomerization is mainly catalyzed by the peptidyl prolyl isomerase activity which facilitates protein folding [478]. CyPs in human are intracellular and extracellular, and they consist of 16 family members having different structures [477]. Among them, CyPA is the most abundant member that was first purified from bovine thymocytes and accounts for ~ 0.1-0.6% of the total cytosolic proteins. It has been shown to be the primary cytosolic binding protein of cyclosporine A [479-481]. Macrophages, vascular smooth muscle cells, endothelial cells, and fibroblast-like synoviocytes secrete CyPA, and it plays a role in both autocrine and paracrine signaling pathways [482-485].

10.2 Role of Cyclophilin in Health:

CyPA stimulates the intercellular communication and is characterized by its chemoattractant influence on inflammatory cells which consequently aggravates

oxidative stress and inflammation [477]. It has been proven that extracellular CyP is a ligand for the surface receptor CD147, and that the binding of CyP to CD147 evidently contributes to its chemotactic activity [486]. It should be noted that the presence of heparan sulfate proteoglycans (HSPGs) is very important in the signaling activity of CyPA because they act as primary binding sites for CyPA on their targets. If HSPGs are removed from the cell surface of target cells (neutrophils), the signaling responses to cyclophilins will be accordingly abolished, and therefore the chemotaxis and adhesion of

T cells and neutrophils will be eradicated [486, 487]. Moreover, it has been shown that CyPA is implicated in protein folding, trafficking, and assembly, in addition to its role in the activation of T cells and cell signaling [477, 488]. It is interestingly related to molecular chaperones because of its enzymatic properties and cellular localization, in addition to its critical role in protein folding [489].

10.3 Role of Cyclophilin in Diseases and IBD:

It has been reported that CyPA is involved in many key processes which underlie different human diseases [477]. Growing body of evidence has shown that an upregulated level of extracellular CyPA is present in patients with inflammatory responses such as asthma [490], sepsis [491], and coronary artery disease [492]. Table 1 shows the involvement of cyclophilin in different pathologies.

Table 1: Cyclophilin's Role in Diseases

	Angiotensin II- induced Abdominal aortic aneurysm (AAA) was prevented by the deletion of CyPA in mice.	[493]
Cardiovascular Disease	CyPA induces ROS production and proliferation of cardiac fibroblasts, thus causing cardiac myocyte hypertrophy.	[494]
	Patients with myocardial infarction and unstable angina have significant high serum CyPA concentration compared to patients with stable angina.	[495]
Rheumatoid Arthritis	CyPA is the major constituent in macrophages of the synovial lining layer in RA.	[496]
	In RA, the destruction of cartilage and bone are mainly caused by CyPA-CD147 interaction which upregulates MMP-9 expression and causes the adhesion of macrophages to the extracellular matrix.	[497]
Diabetes	Plasma levels of CyPA in diabetic patients are much higher than that in healthy individuals.	[498]
Asthma	It was found that asthmatic mice have an elevated level of extracellular CyPA in their airways, and that the airway epithelial mucin is reduced after anti-CD147 treatment.	[499]
Sepsis	The expression of CyPA is elevated in a mouse model, specifically in the liver after sepsis.	[500]
Periodontitis	Inflamed gingival tissues has an elevated cyclophilin expression compared to healthy tissues.	[501]

CyPA plays a role in migration of dendritic cells [502], activation of ERK1/2 MAPK pathway, and NF- κB phosphorylation, therefore causing proliferation of macrophages [503]. It has been further demonstrated that the stimulation of cyclophilin leads to the activation of AKT and NF- κ B pathways, and subsequently to the upregulation of antiapoptotic protein Bcl-2 levels in endothelial cells [504]. Similarly, the binding of cyclophilin to the cell surface receptor CD147 has been shown to increase both ERK and AKT signaling [480, 486, 505]. Thus, the main signaling pathways that are implicated in CyPA/CD147 interactions are ERK1/2, AKT, MAPK, and NF- κB pathways. Furthermore, the association between elevated CyPA levels and cancer has been evidently illustrated [504, 506, 507]. Moreover, CyPA plays a pivotal role in stimulating monocytes to produce matrix metalloproteinases MMP-9 and MMP-2 in inflammatory diseases [508]. The increase of MMP-9 has been interestingly reported in both inflammatory bowel diseases UC and CD [509]. Evident studies have shown that the reduction of inflammation and intestinal mucosal damage in DSS-induced ulcerative colitis mice is mainly caused by the absence of MMP-9 expression [510]. More studies have highlighted the role of tissue inhibitor of matrix metalloproteinase (TIMP-1) in IBD, showing that TIMP-1 level is elevated in IBD patients [511, 512]. It has been evidently shown that the expression of TIMP-1/MMP-9 is regulated by the high expression of serum CyPA in IBD by the activation of ERK1/2 which contributes to the development of IBD, specifically ulcerative colitis [497, 513, 514]. A study has reported a significant increase of CyPA levels in the colonic tissue [515], serum [516], and lymphocytes of UC patients [516] showing that CyPA has a crucial proinflammatory role in IBD.

11. CD147:

11.1 Definition of CD147 and its Role in Health:

CD147, known as ECM metalloproteinase inducer or "EMMPRIN", is a 50-60 kDa transmembrane glycoprotein which is highly glycosylated. It is made up of two extracellular immunoglobulin domains: a transmembrane domain and a cytoplasmic domain containing 39 amino acids [517]. It is mainly expressed on macrophages, endothelial cells, and human peripheral blood cells, in addition to cultured cells. Interestingly, it is implicated in a plethora of cellular processes such as signal transmission, cell adhesion, neural functions, and reproduction.

Additionally, its role has been illustrated in inflammation, production of matrix metalloproteinases, cancer development, and HIV infections [517, 518]. Moreover, EMMPRIN has also participated in transport of calcium [519], chaperone functions [520], development of blood brain barrier [521], and neutrophil chemotaxis. [486] Accumulating evidence has shown that CD147 may bind to integrins. In a study, the colocalization CD147 with □1 integrins has been illustrated in adhesive cellular areas [522]. Well, as mentioned before CD147 has been implicated in several signaling pathways such as: MAPK p38, NF- κB, ERK1/2, and PI3K pathways [523-525].

11.2 Role of CD147 in inflammation and IBD:

It has been proven that CD147 plays an essential role in modulating inflammation and immune responses [526]. Experimental models of human diseases such as rheumatoid arthritis [527], multiple sclerosis [528], asthmatic lung inflammation [499], and myocardial ischemia/reperfusion injury [529] have demonstrated a reduction in inflammation and disease severity when targeting

CD147. Regarding IBD, an elevated expression of CD147 has been detected in the intestinal mucosa of IBD patients.

Similarly, serum CD147 has been also elevated in addition to disease activity (DAI) in these patients. This shows that CD147 is a pivotal proinflammatory biomarker for IBD [530].

12. Syndecan-1/Cyclophilin/CD147 interaction:

Rachel Pakula et al. have investigated the role of Syndecan-1/Cyclophilin "B"/CD147 association in the activation of p44/42 mitogen activated protein kinases and further stimulation of cell adhesion and chemotaxis [531]. Their previous investigations showed that cyclophilin plays an essential role in chemotaxis and integrin-mediated adhesion of T lymphocytes. They have interestingly shown that such processes involve the collaboration with two types of binding sites, CD147 and cell surface Heparan sulfate (HS). In this study, they have demonstrated that only syndecan-1 among other syndecans has a physical association with CD147. They have proven that the addition of antibodies to syndecan-1 or the knockout of syndecan-1 gene have both contributed to a reduction in the Cyp-induced p44/42 activation, and thus a reduction in the migration and adhesion of T cells. Knowing that cyclophilin evokes different responses by a specific mechanism which involves the prolyl isomerization of CD147, findings have shown that syndecan-1 serves as a crucial coreceptor for cyclophilin, and it acts in collaboration with CD147, thus stimulating the activation of p44/42 MAPK pathway, leading to adhesion and migration of T cells. In addition, the pretreatment of T cells with a MAPK pathway inhibitor has consequently inhibited ERK activation, and thus inhibiting Cyp-mediated cell

adhesion to fibronectin. Similarly, the addition of antibodies to CD147 has contributed to the same previous result showing that CD147 has a pivotal role in the signaling pathways triggered by Cyp. This has significantly shown that Cyp evokes ERK activation through CD147 and cell adhesion by a process that involves the functional activity of cell surface heparan sulfate proteoglycan "HSPG" (syndecan-1). Furthermore, it has been hypothesized in this study that a physical interaction between CD147 and HSPG occurs, leading to the formation of an active complex at the cellular membrane of T lymphocytes. Specifically, the association between syndecan-1 and CD147 has been demonstrated despite the absence of Cyp. Well, the presence of Cyp has subsequently contributed to the stabilization or even increase in the association between HSPG and CD147, thus forming an active ternary complex with CD147 at the membrane of T cells. The disturbances in CD147/syndecan-1 complex which occur when adding antibodies to either CD147 or syndecan-1 have shown to neutralize Cyp-mediated ERK activation, thus decreasing the adhesion of responsive T-cells to fibronectin. Knowing that CD147, syndecan-1, and cyclophilin are all incorporated in the inflammatory responses, data in this study have suggested that the interaction between the three subunits may have a pivotal role in the pathogenesis of several inflammatory diseases including IBD.

13. Probiotics:

13.1 Definition of Probiotics:

Probiotics are active nonpathogenic living microorganisms which confer positive effects to the host when consumed in sufficient doses [532]. Their efficient

role has evidently been demonstrated in the treatment of several human diseases [533-535]. It has been shown that probiotics have specifically beneficial effects in treating inflammatory diseases such as arthritis [536], ulcerative colitis [537, 538], and experimental colitis [539-541].

13.2 Mechanisms of Action of Probiotics:

No doubt that the effect of probiotics mimics that of the intact microbiota during intestinal homeostasis. Their mechanism of action first of all depends on their strain and involve the assembly of antibacterial components such as bacteriocins, lactic acid, and hydroperoxides. Second, they play a crucial role on the intestinal epithelial surface where they have the ability to block completely the epithelial binding sites, thus preventing bacteria from binding. Third, they fortify the integrity of the mucosal barrier by upregulating the tight junction molecules. Also, they have the ability to destroy toxin receptors and modify pH, thus creating a more acidic milieu which is unfavorable for the survival of proinflammatory bacteria. Finally, they tend to compete for indispensable nutrients that are needed for the survival of the host [207, 542-544]. Moreover, probiotics are very well known for their pivotal role in regulating immunity and assisting in the defense mechanism of the immune system. Actually, they are involved in the activation of Toll-like receptors and differentiation of T-helper cells, and the production of mucosal antibodies (IgA). Furthermore, they play a vital role in a plethora of mechanisms. They stimulate phagocytosis and natural killer (NK) activity and provoke apoptosis of T cells. They also decrease proinflammatory cytokines (TGF-α, IFN-γ), and increase the secretion of anti-inflammatory cytokines (TGF-ß, IL-10) [545-547].

13.3 Probiotics' Effectiveness in Clinical Cases:

Interestingly, the effect of probiotics in the reduction of inflammation has been established in various clinical studies. For instance, Li et al. have revealed the essential role of Lactobacillus acidophilus, Streptococcus, and Bifidobacterium bifidum in the downregulation of the proinflammatory cytokine IL-1 and upregulation of IL-10 which is a well-known anti-inflammatory cytokine [548]. Additionally, Chen et al. have highlighted the important role of Escherichia coli Nissle 1917 and VSL#3 which is mainly a food product that involves (L. paracasei, L. plantarum, L. acidophilus, L. delbrueckii, B. longum, B. breve, B. infantis, and Streptococcus thermophilus) in having a positive impact on UC patients [549]. Moreover, it has been revealed that the supplementation of Bifidobacterium-fermented milk (BFM) causes a reduction in the concentration of a molecule which is involved in colitis remission (Luminal Butyrate), showing an improvement in the function of colorectal mucosa [550].

13.4 Probiotics' Effectiveness in Animal Models of Colitis:

A study has demonstrated the effect of a seven-days pretreatment with L. plantarum DSM 9843, Bifidobacterium sp. 3B1 and B. infantis DSM 15158 in a DSS-model of colitis. Accordingly, a reduction in both disease activity and bacterial translocation have been evidently revealed in rats [551]. Another study by McCarthy et al. have shown the effect of Lactobacillus salivarius and Bifidobacterium infantis strains in the IL-$10^{-/-}$ model where a significant reduction in the pro-inflammatory cytokines TNF-α, IL-12, IFN-γ has been detected, consequently leading to a reduction in the mucosal inflammation [552]. Similarly. Madsen et al. have validated the effect of VSL#3 in the IL-$10^{-/-}$ mice model where

a significant reduction in the pro-inflammatory cytokines (TNF-α and IFNγ), colitis severity, and histological scores have been detected as well [553].

Moreover, Fujiwara et al. have shown the effect of Bifidobacterium longum in a DSS-mouse model of colitis where a reduction in colonic shortening and disease severity have been revealed [554]. Another study has shown that the supplementation with other strains of Bifidobacterium such as B. animalis subsp. lactis BB12 has caused a reduction at the level of colon length and colon's histology, in addition to the decrease in TNF-α levels and in the apoptosis in intestinal epithelial cells [555]. Interestingly, it has been proven that probiotics have a pivotal role in supporting and intensifying gut-tight junctions, in addition to affecting the proportions of T cell subpopulations. It has been shown that the mixture of Bifidobacterium, Lactobacillus acidophilus, and Enterococcus in a DSS-model of colitis has subsequently contributed to a reduction in the total number of T cells in the colon and peripheral blood, an increase in the number of T reg cells, and strengthening in the gut-tight junctions [556]. In other models of colitis such as TNBS models, it has been demonstrated that the oral treatment with Bifidobacterium bifidum has reduced the inflammatory state, histological scores, and macroscopic damage, and it has avoided weight loss of the animals [557, 558]. However, no beneficial impact has been detected at the levels of histological scores, weight changes, and gut permeability in TNBS rat models of colitis after the supplementation of Lactobacillus plantarum species 299 in contrast to other studies and reports. This may be to several factors including the used dose, the severity of the disease, the animal model of colitis (mice or rats), and the probiotics' strains

used which may have different properties affecting their mechanisms of action [559].

14. Cluster of differentiation 3 (CD3):

14.1 Definition of CD3:

Cluster of differentiation 3 or CD3 is a cell surface multimeric protein complex, identified as a T3 complex. It mainly constitutes of four different subunits; epsilon, gamma, delta and zeta, where they dimerize and form three distinct pairs. These subunits are chains of integral membrane glycoproteins that have a non-covalent association with the T cell receptor (TCR). They are necessary for TCR cell surface expression and signaling transduction as well [560-562]. It has been shown that the expression of CD3 has initially taken place in the cytoplasm of the developing T cells. As the T cell maturation process progresses, the cytoplasmic CD3 is subsequently abolished, and CD3 antigen is only presented on the cell surface [561]. At the pathophysiological level, the binding of antigen peptides leads to the stimulation of TCR, thus evoking the phosphorylation of (ITAMS) which are intracellular immunoreceptor tyrosine-based activation motifs found in the CD3 subunits [562].

14.2 CD3 in Diseases and Inflammation:

CD3 antigen has evidently been demonstrated as an ultimate immunohistochemical T cell marker in tissue sections to distinguish between normal T cells and T cell neoplasms such as lymphomas and leukemias [563, 564]. Additionally, CD3 has been interestingly shown as a marker for various diseases such as collagenous colitis [565], lymphocytic colitis [566], and coeliac disease [567]. Moreover, Takeuchi et al. have proven the association between the

deficiency in the CD3 polypeptide chain and the development of Systemic Lupus Erythematosus (SLE), an auto-immune disease [568].

Furthermore, deficiencies in CD3 have been linked with the development of "Severe Combined Immune Deficiency" (SCID) where T cells are either defective in their function or production [569, 570]. It has been also demonstrated that the addition of antibodies specific to CD3 in animals has consequently stimulated tolerance to allografts [571].

In a murine model of "Pneumocystis Pneumonia", it has been proven that anti-CD3 antibody treated to mice has evidently reduced inflammation after one week, and improved their health consequently compared to mice receiving control antibody [572].

Another study has revealed the association between the incubation of intestinal tissues from IBD patients with "Otelixizumab", an anti-CD3 antibody, and the reduced production of pro-inflammatory markers (IL-17A, IFN-γ). In contrast, it has been shown that an increased production of anti-inflammatory cytokine IL-10 has been detected posttreatment with anti-CD3. This indicates that CD3 is an inflammatory marker that is upregulated during inflammatory diseases, including "IBD" [573].

15. Phosphorylated Protein Kinase B (pAKT) in Health and Diseases:

The PI3K/Akt pathway is one of the crucial pathways that control several mechanisms such as cell proliferation, differentiation, and survival by inhibiting

other apoptotic processes. It has been evidently shown to be activated in various types of cancers [574-578]. Phosphatidyl inositol bisphosphate (PIP2) is converted to phosphatidyl inositol trisphosphate (PIP3) by the action of PI3K enzyme. Subsequently, the formed (PIP3) translocates Akt to the plasma membrane where its phosphorylation process takes place by the action of 3-Phosphoinositide-dependent kinase1 (PDK1) enzyme [579]. Thus, the activated phosphorylated form of Akt (pAkt) is formed which further modulates the function of a plethora of substrates incorporated in the regulation of cell growth and survival.

It has been demonstrated that Akt signaling controls the differentiation of Th1 cells [580, 581]. Further studies have proven that the activation of PI3K/Akt/mTOR pathway in the lymphocytes of murine models has displayed symptoms of systemic autoimmunity, showing the critical role of this pathway and its involvement in autoimmune disorders [582]. Moreover, it has been shown in one of the studies that "Beauvericin" ameliorates experimental colitis in mice through the inhibition of activated T lymphocytes by downregulating the PI3K/Akt signaling pathway. Hence, this pathway is upregulated in mice inflammatory bowel disease [583]. Another study has shown that its activation is also involved in human IBD as well as tumor invasion in the Piroxicam / IL-10−/− mouse model. The use of LY294002, a mast cell and tumor-associated macrophages (TAMs) inhibitor, has targeted the PI3K/Akt pathway, consequently impeding the progression of colitis. This indicates that the development of colitis and progression to cancer depend on the activity of stromal PI3K [584]. A study has revealed that fibrinogen stimulates vascular permeability through Akt activation and consequent microfilament depolymerization. A significant increase in the expression of phosphorylated Akt

(pAkt) has been shown in the colons of DSS and TNBS-induced mice models of colitis [585]. Therefore, PI3K/Akt pathway is a therapeutic target for IBD.

16. Beta-1 integrins and IBD

The underlying pathogenesis of IBD is tightly regulated by a stepwise process involving multiple integrins molecules, which have become prominent molecular players in IBD research and clinical management [586].

Multiple studies documented the central role of integrins, which are heterodimeric transmembrane receptors bridging between the extracellular matrix and the cytoskeleton, in the recruitment of immune cells, thus leading to an increase in guthoming lymphocytes. Actually, integrins serve as cellular keys to direct lymphocyte trafficking into intestinal and non-intestinal tissues as well as adhesion, signaling and proliferation [587].

The integrins family consists of 18 alpha and 8 Beta subunits forming a combination of 24 different receptors which bind specific ligands. They play an essential role in multiple physiological and pathophysiological processes. They maintain essential balance between apoptosis and cell proliferation in normal cells, thus regulating cell migration, cell proliferation and even inhibiting cell death. In IBD, many of the key cell-cell and cell-matrix interactions are regulated by integrins, whose deficiency may significantly enhance both pathogenesis and development of IBD [588].

Beta-1 integrins can heterodimerize with alpha-4 and get expressed on most leukocytes including neutrophils, memory and effector lymphocytes. They are highly expressed on lamina propria lymphocytes (LPL) and contribute to T cell

responses by providing costimulatory signals [588]. The high expression of Beta-1 integrins on CD4+memory cells of gut mucosa, as a result of co-stimulation, restores CD3-induced proliferation of CD4+LPL and reduces the activation induced apoptosis. Such stimulation increases the capacity of the LPL's to express proinflammatory cytokine transcripts [587].

In IBD, Beta-1 integrins are highly expressed on CD4+ memory T cells of inflamed gut mucosa of UC and CD patients. They modulate the response of LPL's in intestinal inflammation after TCR stimulation [588]. Such an increase in expression of activated Beta-1 integrins on LPL in intestinal inflammation could perpetuate the inflammatory process from acute to chronic.

Consequently, Beta-1 integrins activate multiple signaling pathways via Ros/MEK/ERK/PISK and AKT leading to cell proliferation and survival. They have also been involved in activating TGFB, in particular, in Chron's Disease, supporting the over expression of its downstream related genes. For example, the up-regulation of Beta-1 integrins induces p38/MAPK signaling during the EMT process [586] which can occur in IBD.

In brief, the Beta subfamily, in particular Beta-1, also known as very late activation (VLA) receptors, binds to various ECM glycoprotein ligands such as fibronectin, laminin, collagen and tenascin. As a result, especially when highly expressed, they facilitate, through a number of signaling pathways, proliferation and adhesion of lymphocytes to matrix components, and increase the gene transcription of inflammatory mediators as well as several ECM-modulating enzymes, thus playing an essential role in the pathogenesis and perpetuation of inflammatory disorders like IBD's.

A common complication of IBD is a progressive stricture formation, narrowing and sometimes stenosis of the intestine resulting from chronic tissue damage and fibrosis. Integrins are key players in the onset of such IBD associated fibrosis, as a result of the persistent interaction between cellular compartments and the ECM through mechanotransduction. This mechano-signaling is a well-regulated process, that depends on many proteins, including integrins, leading to matrix stiffness and tissue dysfunction (V. Garlatti et.al. 2021). Actually, Integrins and the downstream focal adhesion (FAK) complex proteins function as mechano-sensors, induce downstream signaling pathways in response to matrix stiffness. In fact, there is a close relationship between the matrix, which exerts force on the cell via integrins, and the cellular cytoskeleton, which in turn resists this force. Integrins can also potentiate the action of TGFB pathway, supporting the over expression of its downstream related genes and its interaction with Smad3, alpha-V Beta-1 mTor during fibrogenesis. Over expression of alpha 3 Beta-1 enhances MMP9 expression and induces p38/MAPK signaling during the epithelial-mesenchymal transition (EMT) process. Moreover, a functional role for the fibroblast expressed integrins, including alpha-V Beta-1 has been documented in fibrosis working as mechano-transducers [586].

Consequently, blocking alpha-4 Beta-1, among others, was a target for IBD treatment and led to significant reduction in colitis. The efficacy of these pharmacological therapies targeting integrins relies on their capacity to target integrin-mediated recruitment and functionality of immune cells at the damage site. However, the role of integrins in the pathogenesis of IBD could be in the context of both immune-dependent and independent mechanisms [586].

CHAPTER II
HYPOTHESIS AND OBJECTIVES OF THE STUDY

The treatment modalities of inflammatory bowel diseases (IBD's) have evolved deeply since the discovery and use of cyclosporine A in the seventies as an immunosuppressant.

Several studies have shown that cyclosporine "A" plays a role in the therapy of IBD in both clinical and animal models [444, 469, 472-474]. However, as previously mentioned, various systemic adverse effects have been reported in treated patients and animals [476]. Other studies have highlighted the prominent role of Syndecan-1/Cyclophilin "A"/CD147 interactions in inflammation and their possible role in the pathogenesis of IBD [589]. It is hypothesized that the combination of CypA and CyA might decrease the side effects of CyA, particularly in the presence of probiotics, since research studies have evidently revealed the positive effect of probiotics on the course of IBD in reducing colonic edema, macroscopic damage, histological alterations, and clinical features in both human and animal models. On this basis, we hypothesized that:

- Cyclosporine "A" would reduce inflammation in syndecan-1 knock out mice IBD

models due to its proven immunosuppressive therapeutic effects in IBD models.

- Cyclosporine "A" and cyclophilin A treatment would have an added value to improve the healing process in IBD with limited side-effects.
- Cyclophilin A is a potential treatment to decrease inflammation in IBD.
- Probiotics would have a positive effect on IBD in syndecan-1 knock outs in ameliorating inflammation and speeding up the healing process.

Accordingly, this project was based on four objectives:

- To evaluate the independent effect of cyclosporine “A” and cyclophilin A on the expression of several inflammatory markers (CD147, IL-6), ß1 integrins, CD3 and pAKT in Syndecan-1 knock out mice and wild type IBD models.
- To assess the anti-inflammatory activity of a combination of cyclosporine “A” in the presence of extracellular cyclophilin “A” in Syndican-1 knock out and wild

  type mice with DSS induced IBD.
- To explore the effect of probiotics on IBD alone and in presence of (CypA -CyA) complex in Syndican-1 knock out mice as well as wild type.
- To determine the differential effect of both (CypA-CyA) on the various markers tested.

Data emanating from this research will probably clarify and highlight the appropriate use of cyclosporine A, cyclophilin A and Probiotics in the management of IBD.

CHAPTER III
MATERIALS AND METHODS

1. Animals

A total of 112, 6-8-week-old, mice were divided into 2 main groups: Wild type male Balb/c mice and Knock out Syndecan 1 null male mice (56 mice of each strain). All the animals were housed in the Animal Care Facility of the American University of Beirut (AUB). They were kept at a constant temperature ranging between 18ºC and 22ºC and humidity between 40 and 60 % with an alternating light cycle 12 hours light – 12 hours dark. Animal feed and water were provided at libitum. All animal experiments and procedures followed strictly the guidelines of the Institutional Animal Care and Use Committee (IACUC) at the American University of Beirut for the care and use of laboratory animals.

2. Experimental design

16 control mice – 8 from each strain were only provided with normal drinking water and intraperitoneal (IP) saline injections. IBD was induced in 48 animals from each strain (96 mice) by 2 % DSS in drinking water. Each DSS cycle consisted of 7 days of DSS followed by 2 weeks normal drinking water. At week 3, the animals were sacrificed.

The 96 mice with DSS induced IBD were divided according to the different treatment modalities into a total of 12 subgroups (8 animals per group).

Treatments were administered as illustrated in Table 2.

Table 2: various groups and corresponding treatments:

Balb/C mice - control group given normal water	Syndecan 1 null mice - control group given normal water
Balb/C mice + DSS only	Syndecan 1 null mice + DSS only
Balb/C mice + DSS + Cyclophilin A	Syndecan 1 null mice + DSS + Cyclophilin A
Balb/C mice + DSS + Cyclosporine A	Syndecan 1 null mice + DSS + Cyclosporine A
Balb/C mice + DSS + Cyclophilin A + Cyclosporine A	Syndecan 1 null mice + DSS + Cyclophilin A + Cyclosporine A
Balb/C mice + DSS + Cyclophilin A + Cyclosporine A + Probiotics	Syndecan 1 null mice + DSS + Cyclophilin A + Cyclosporine A + Probiotics
Balb/C mice + DSS + Probiotics	Syndecan 1 null mice + DSS + Probiotics

3. Induction of IBD and Treatments' preparation and administration

DSS-induced IBD is a well-established model commonly used in experimental colitis studies. Optimized concentration of the pro-inflammatory agent Dextran Sodium Sulfate (DSS-Sigma-Aldrich,42867-100G) 2 % was prepared in autoclaved water and administered to animals in their drinking water. Each DSS cycle consisted of 7 days of DSS followed by 2 weeks normal drinking water. The animals were divided into seven groups and treated as follows:

Cyclophilin A (Human recombinant expressed in E-coli) from Sigma - Aldrich (C3805- 1MG) was injected intraperitoneally (IP) at a dose of 25µg /kg/Day for one week starting day 7 of DSS administration. Similarly, Cyclosporine A (Novartis, SPE31) was administered by IP injections at a concentration of 200µg every other day for 2 weeks starting day 7 of DSS treatment. In addition, probiotics

(P) used was a mixture of 7 strains of lactic acid-producing bacteria: *lactobacillus rhamnosus, saccharomyces boulardii, bifidobacterium breve, bifidobacterium lactis, lactobacillus acidophilus, lactobacillus plantarum and lactobacillus reuteri.* One capsule of (P) was dissolved in 1.75 L of autoclaved tap water to reach a daily dose of 10^8 CFU per animal given for 2 weeks starting day 7 of DSS treatment.

4. Clinical course assessment:

During the experimental period, the animals were monitored for clinical symptoms and signs including body weight, stool aspect, and rectal bleeding. Scores were recorded throughout the experiment based on the parameters: gross bleeding (0= absence; 2=blood stained; 4= clear presence of blood), stool consistency and watery diarrhea (0= normal, 2= loose, 4= diarrhea), weight loss (0= normal; 1=1-5%; 2=5-10%; 3=10-20%; 4 more than 20%), and a previously validated clinical disease activity index (DAI) which assessed weight loss, diarrhea, fur, gross bleeding and posture with a range of 0 to 4 was calculated [590].

5. Measurement of fecal occult blood

Collection of feces was done by placing a single mouse in an empty cage without bedding material for few minutes; feces were collected and Occult blood was measured using HemoCue America Beckman Coulter™ Hemoccult™ Fecal Occult Blood Slide Test System, as per the manufacturer instructions [591].

6. Dissection, colon length measurment and biopsy removal:

After 3 weeks, on the experiment endpoint, animals were sacrificed by isofurane overdose and cervical dislocation then dissected in order to remove their colon. We measured and recorded the length of each isolated colon from ileocecal valve to rectum, then it was quickly flushed on ice with phosphate-buffered saline (PBS) to clean it. A portion of this clean colon, the sigmoid, was fixed in 10% buffered formalin for routine histological processing. The other portion of the sigmoid and the descending colon were frozen and kept in liquid nitrogen for further molecular studies.

7. Histology

Routine processes were used for the microscopic analysis. The proximal and distal sigmoid colon tissues were removed and fixed in 10% formalin for 48 hours. Then formaldehyde fixed tissues were dehydrated using a series of ascending alcohol concentrations then embedded with paraffin. Blocks were sectioned on a microtome, 5 µm-thick sections and mounted on slides pre-treated with tissue adhesive solution. Seven slides of each biopsy were prepared, one slide was stained with water-soluble hematoxylin and eosin (H&E) to investigate the histopathological changes that might be detected at the level of the intestinal layers. Periodic Acid Schif (PAS) stain was also used to examine mucus and goblet cells. The slides were finally scanned using a Light Microscope. The histological score of each animal within each group was assessed and calculated based on a

histological scoring system shown in Table 3 [592]. The different sections were photographed using Olympus CX-41 microscope.

Table 3: Scoring system used to evaluate the histological alterations in dextran sulphate sodium (DSS)-induced colitis. [592]

Feature	Score	Description
Severity of inflammation	0	None
	1	Mild
	2	Moderate
	3	Severe
Extent of inflammation	0	None
	1	Mucosa
	2	Mucosa and Submucosa
	3	Transmural
Crypt damage	0	None
	1	1/3 damaged
	2	2/3 damaged
	3	Crypts lost, surface and epithelium present
	4	Crypt and surface epithelium lost

8. Western Blot

Western blot: Western blotting analysis was performed according to standard protocols. Briefly, 100 mg of colon tissue homogenized with Laemli lysis buffer, centrifuged at 10,000 rpm for 10 min at 4ºC, supernatant was collected and kept in aliquots. Protein concentration was determined by the Lowry method using the DC™ Protein Assay Kit (#5000111). For gel loading, protein samples were boiled with loading buffer for 5 min at 95ºC and separated in SDS PAGE. Proteins

were transferred to nitrocellulose membranes (Bio-Rad Laboratory, CA, USA). Tricolor Broad Protein Ladder (3.5-245 kDa) was used from Abcam (K00059-0250). The blots were then blocked with 5% BSA in Tris-buffered saline for 1 hour at room temperature, and then incubated overnight at 4°C at a dilution of 1:500 with their respective primary antibodies. Antibodies against IL-6 (anti-mouse SC-57315), CD147 (anti-mouse SC-46700), Actin (anti-mouse SC-47778), CD3 (anti-mouse 20047), and pAKT (anti-mouse SC57315) were purchased from Santa Cruz Biotechnology. Then, the primary antibodies were detected using a secondary antibody (horseradish peroxidase-conjugated antimouse; Abcam 97046 at a dilution of 3:40000), in which membranes were incubated for 1 hour at room temperature. The membranes were rinsed with TBST before and after the incubation with the secondary antibody. Finally, the immunoprecipitated protein bands were detected with ChemiDoc MP Imaging System-Biorad.

9. Immunohistochemistry

Immunohistochemistry was performed on 5-micron thick paraffin embedded sections that were obtained by microtome cutting. For antigen retrieval, slides will be immersed in citrate buffer (pH 6). After washing with TBST (pH 7), the slides were stained with a primary antibody (anti-integrin ß1- JB 1B sc- 59829 mouse IgG from Santa Cruz Biotechnology) at 4 °C overnight. After washing the slides 3 times with TBST, the sections were incubated with the appropriate (coupled with alexa fluor 594) secondary antibody (m IgG Bp FITC conjugated from Santa Cruz Biotechnology) in TBST with 5% BSA for 2 hours at room temperature. The nuclei were counterstained with ProLong™ Diamond Antifade Mountant with 4',6-diamidino-2-phenylindole (DAPI) (Product # P36962), and sections were

photographed using Zeiss Axio Observer-Z1. Quantification was done by obtaining the measurement of mean fluorescence intensity (MFI) of integrin ß1 in a region of interest (ROI) and calculating the integrated density using Zen 2.3 Software.

10. Dihydroethidium (DHE)

DHE was performed on frozen tissues, colon rings were demarcated with a solvent resistant pen. 1/1000 DHE solution (Thermo Fisher# D11347) was prepared and dispensed over the tissue and the slides placed for 30 min at 37°C. Then, the DHE residues were removed, slides counterstained with DAPI, a cover slip put on and stored at 4°C (light sensitive) until microscopic evaluation and quantification using Zen 2.3 software.

11. Statistical Analysis

Statistics were being performed using GraphPad Prism 8.0.1. Data were expressed as a mean ± standard deviation. Significant differences were evaluated using the oneway ANOVA by Tukey_Krammer multiple comparisons test. A value of $P < 0.05$ was considered significant.

CHAPTER IV
RESULTS

1. Macroscopic assessment

1.1 Clinical symptoms and signs:

DSS administration induced, in both strains, sever inflammation in the bowel as a result of one-week treatment. Group One, the normal control, without DSS treatment, showed no symptoms and signs of IBD: no diarrhea, no hematochezia and normal growth in weight. On the other hand, mice in both strains, in group two treated with DSS, showed severe diarrhea and hematochezia, more so in the knock out mice (Table 4).

In wild type group 2, when DSS was used alone, 6 out of 8 animals showed severe diarrhea and 5 suffered from hematochezia. On the other hand, 8 out of 8 animals of the syundecan-1 KO mice showed diarrhea and 6 mice suffered from hematochezia (Table 4).

When cyclophilin A (Cyp A) was added alone, group 3, hematochezia and diarrhea were both present in 5 mice out of 8 in the wild type mice while 7 animals of the syndecan-1 KO mice showed diarrhea and 5 suffered from hematochezia (Table 4).

Cyclosporine (CyA) alone, group 4, reduced the hematochezia in the wild type from 5 to 3 mice only and diarrhea from 6 to 4 mice. However, group 4 in the syndecan1 KO mice diarrhea was present in 3 mice and hematochezia in 4 mice compared to 6 mice in group 2, DSS alone (Table 4).

However, treatment with the CypA-CyA combination, group 5, showed

hematochezia in 3 mice instead of 5 in the DSS only group in the wild type, but no effect was noted on diarrhea which remained in 6 mice. On the other hand, in the combination of CypA-CyA hematochezia was still detected in 4 mice and diarrhea in 3 mice of the KO mice (Table 4).

When adding probiotics to the combination, group 6, 2 mice showed hematochezia in the wild type compared to 5 mice in Group 2, given DSS only, and 4 mice suffered from diarrhea. On the other hand, 3 KO mice suffered from hematochezia and 3 from diarrhea when given the CypA-CyA combination plus probiotics (Table 4).

Treatment with probiotics alone, in mice with DSS induced IBD, stopped the bleeding in all mice from both strains and reduced diarrhea to 2 animals only in the wild type and 2 as well in sydecan-1 KO mice (Table 4).

As for the DAI, the highest disease activity indices were obtained in animals belonging to the DSS only group in both strains. It is important to note that, treatment with probiotics alone improved the clinical profile and decreased the DAI to almost 0 in the wild type mice. On the other hand, in Syndecan-1 KO mice, DSS alone and DSS plus combination of cyclosporine and cyclophilin showed the highest DAI, and mice were shown to present more signs of discomfort and distress.

Table 4: Comparative assessment of clinical symptoms and signs

	Wild type Balb/C		Syndecan-1 KO	
Groups	Diarrhea	Hematochezia	Diarrhea	Hematochezia
Group 1 no DSS	0	0	0	0
Group 2 DSS only	6	5	8	6
Group 3 DSS + Cyclophilin	5	5	7	5
Group 4 DSS + Cyclosporine	4	3	3	4
Group 5 DSS + CypA + CyA	6	3	3	4
Group 6 DSS + CypA+CyA + probiotics	4	2	3	3
Group 7 DSS + probiotics	2	0	2	0

1.2 Shortening of the colon:

As a result of the inflammatory process in the pathogenesis of IBD, structural alterations take place in the colon at molecular, microscopic and eventually macroscopic levels. Such structural changes manifest most of the times through strictures, granulomas, appearance of pseudopolyps, as well as shortening of the colon. The more chronic the inflammation, the more severe will be the abnormal shortenings of the colon.

After the three-week duration of the experiment, the length of the colon did not go through significant shortening in both strains of mice. Such shortening was decreased in the presence of probiotics, be it in combination with the CypA-CyA complex or even better with probiotics alone in both strains.

DSS treatment shortened similarly the colon by a ratio of 8/10=20% in Sydecan1 knock out mice and 11.2/13.4=16.5% in the wild type compared to the

controls (Figure 1). CyA and CypA did not prevent this shortening; they actually enhanced it slightly and more so when present in combination without probiotics, in particular in the wild type. However, the shortenings were only slightly significant when compared to the control non-treated group, in particular, in the wild type.

In brief, probiotics prevented to a great extent such shortenings and more so in the wild type, when the shortening was more obvious, be it used alone or in combination with the CypA-CyA complex.

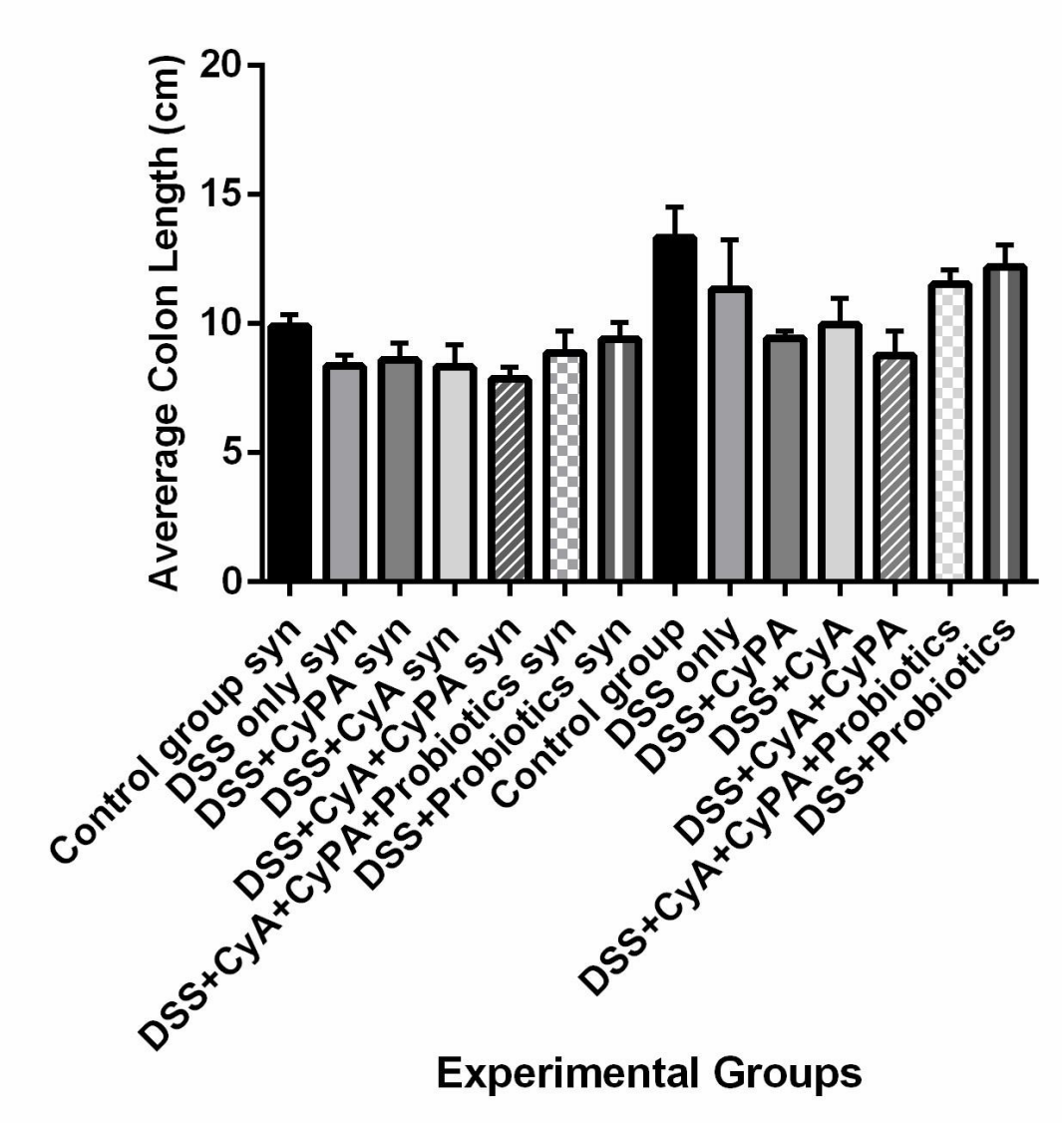

Figure 1. Colon Length variation at the day of sacrifice.

2. Microscopic assessment *2.1 Effect of Cyclophilin A, Cyclosporine and probiotics on colon histology in* Balb/C and Syndecan-1 knockout mice

In IBD, colonic inflammation involves a series of structural and pathological changes that are reflected by the disruption of the architecture of colonic mucosa resulting in ulceration, epithelial denudation and infiltration of a massive mix of inflammatory cells into the mucosa and submucosal layers.

In order to examine mucosal inflammation and evaluate the effect of Cyclosporine (CyA), cyclophilin A (CypA), probiotics and their combination (CyA+CypA; CyA+CypA+P) on mucosal modulation and inflammatory cell infiltration, histological analysis of H&E stained sigmoid tissues was performed for both strains separately, then a comparison between the two strains followed.

Concerning the wild type, as shown in Figure 2A, the histological architecture of the colon in the healthy control group 1 showed no sign of disrupted morphology and a normal histological appearance.

In contrast, the DSS-treated group 2 (Figure 2B) exhibited an extensive inflammatory cell infiltration that invaded the mucosa and submucosa and reached the muscular layer. A disruption of the crypts' integrity and a loss of epithelial lining were also observed (Figure 2B). Similarly, the group treated with CypA displayed high inflammatory cell infiltration, disorganized crypts, and loss of the majority of the epithelial lining (Figure 2C). However, colonic inflammation subsided substantially in the groups treated with either of CyA (Figure 2D), probiotics (P) (Figure 2F), and their combination (CyA+CypA; CyA+CypA+P) (Figure 2E and 2G, respectively). In those groups, histological damage was

progressively reduced compared to the DSS-treated control group. The mucosal architecture, the crypt integrity and the epithelial lining were restored to a great extent. Inflammatory cells activity was identical in certain areas, however significantly reduced compared to the DSS-treated group 2.

Histological scoring confirmed the above-mentioned observational findings indicating that inflammation was most significantly alleviated in groups treated with the combinations (CyA+CypA+P or CyA+CypA), probiotics and cyclosporine, compared to those in DSS and cyclophilin treated groups (Figure 6).

The results suggest that probiotics, cyclosporine and the combination (CyA+CypA+P; CyA+CypA) might have a beneficial effect in reducing mucosal damage in DSS-induced colitis mouse model, while cyclophilin A alone might provide an exacerbating effect (Figure 6).

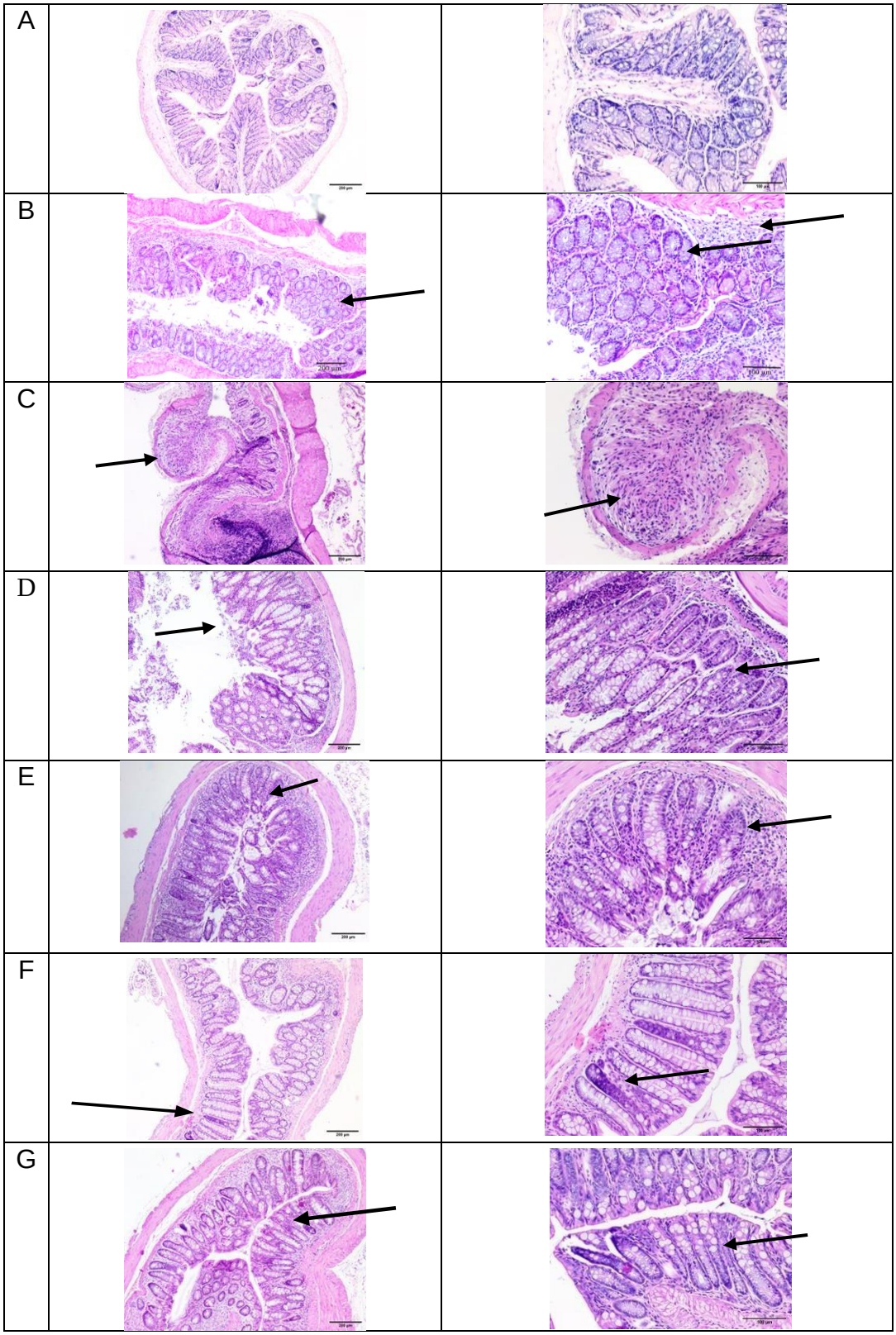

Figure 2. Wild type mice: Effect of Cyclophilin A, Cyclosporine A and probiotics on colon histology (H&E). Slides were stained with H&E and photographed at 40x magnification, scale bar 200 micron (left column) and 100x, scale bar 100 micron (right column). Arrows indicate reaction sites.

Concerning the Syndecan-1 knockout (Synd-1 KO) mice, shown in (Figure 3A), the negative control group 1S (no DSS), colon tissues are morphologically normal, showing vertically oriented crypts lined by columnar epithelium. All intestinal layers are preserved and the leukocyte infiltration is within the normal range. In contrast, the DSS group 2S (Figure 3B) suffered a severe inflammation extending from submucosa to mucosa layers with the presence of a marked edema between muscular layer and submucosa. A massive increase of immune cell infiltrate and a complete loss of epithelial architecture are both observed. However, some portions of the section are characterized by semipreserved crypts with a low leukocyte infiltration; a spotted inflammation characteristic of Crohn's disease (Figure 3B).

On the other hand, mice treated with (DSS+CypA) group 3S and (DSS+CyA) group 4 exhibited a significant improvement in colonic histology showing a mild inflammation compared to the group treated with DSS only group 2S (Figure 3C, 3D). Only a one-third damage of the crypts is present with a very low level of edema between muscular layer and submucosa. The overall architecture is almost preserved and a low level of leukocyte infiltration localized in the mucosa and submucosa layers with a little more in the DSS+CyA group where lymphocytes aggregates are very active (Figure 3D).

In contrast to the (DSS+CypA) and (DSS+CyA) separately treated groups 3S and 4S, the treatment with (CypA-CyA) complex group 5S along with DSS caused a detrimental effect on the tissue architecture where two–third of the crypts are lost in addition to the intensive leukocyte infiltration spreading throughout the

mucosa and submucosa. The thickening of the mucosa and submucosa layers is significant, as well as the notable presence of areas of complete epithelial denudation (Figure 3E).

On the other hand, a slight reduction in the severity of inflammation is clearly observed after the addition of probiotics to (CypA-CyA complex) along with DSS (Figure 3F). The inflammation is still invading the mucosa and submucosa layers and even between muscular layer and submucosa compared to the group treated with DSS+(CypACyA) complex. The heavy infiltrates are mainly localized between the muscular layer and submucosa layers and also in the mucosa. The crypts are partially preserved, only onethird is distorted with very active Lymphocytes aggregates (Figure 3F).

However, group treated with (DSS+probiotics) only showed a marked reduction in inflammation in the absence of (CypA-CyA) complex (Figure 3G). About only one-third of the crypts are inflamed while the remaining crypts are preserved. A moderate leukocyte infiltration is present, probably at Peyer's patches. However, the remaining parts of the tissue (3/4) reflect a normal morphological image with very low levels of infiltrates.

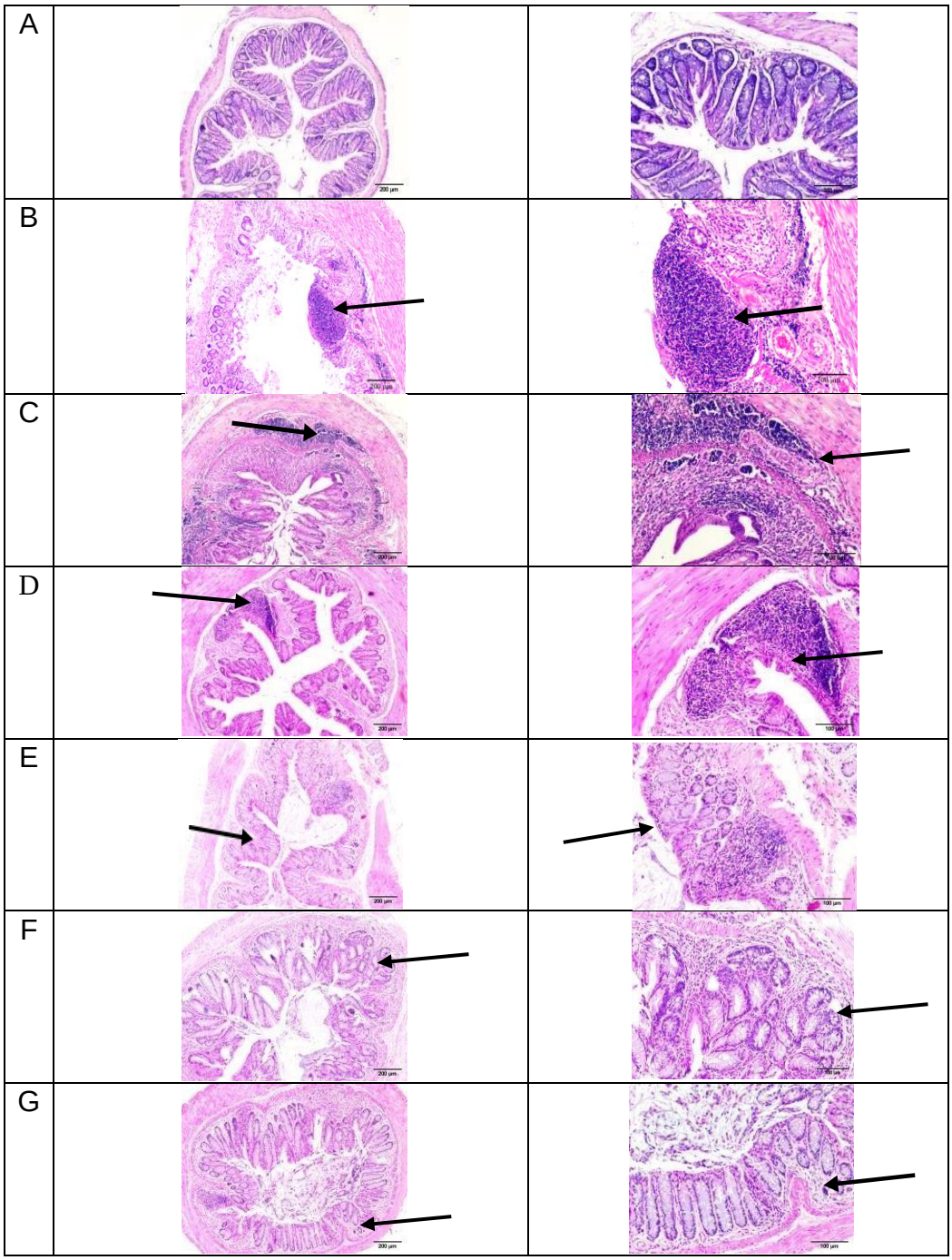

Figure 3. Syndecan-1 Null mice: Effect of Cyclophilin A, Cyclosporine and probiotics on colon histology (H&E). Slides were stained with H&E and photographed at 40x magnification, scale bar 200 micron (left column) and 100x, scale bar 100 micron (right column). Arrows indicate reaction sites. (A) Negative control group (no DSS) showing normal colon morphology with normal crypt architecture; (B) DSS-treated group showing a marked localized inflammation invading mucosa and submucosa and characterized by edema, massive increase of leukocyte infiltration. However, semi-preserved crypts are present throughout the remaining areas with low infiltration. (C) DSS+ CypA treated group showing a mild inflammation with an improvement in the colonic histology, preservation of overall architecture, and low leukocyte infiltration. (D) DSS+ CyA treated group showing a mild inflammation with only a one-third crypt damage and very active Peyer's patches. (E) DSS+(CypA-CyA) complex treated group showing a detrimental destruction of the complete colonic morphology and crypt architecture with an intensive leukocyte infiltration. (F) DSS+(CypA-CyA) complex+ probiotics treated group showing a moderate inflammation extending from submucosa to mucosa with a one-third damage of the crypts. A heavy leukocyte infiltration is restricted to the space between muscular layer and submucosa in addition to mucosa. (G) DSS+ probiotics group showing a marked reduction in inflammation where only one-third of the crypts is distorted. However, a moderate leukocyte infiltration is concentrated at Lymphocytes aggregates while the remaining areas reflect a normal morphology with a low infiltration.

2.2 Assessment of mucus secreting cells, Goblet cell, by Periodic Acid Schiff stain (PAS):

The use of PAS is to demonstrate mucopolysaccharide moieties, in particular, the mucus in the goblet cells.

The negative control group in wild type mice displayed a typical tissue architecture with an abundance of goblet cells spread across the colon tissue (Figure 4A). On the other hand, the group that received DSS showed a great loss of goblet cells (Figure 4B) and change of their architecture. Obviously, the effects of DSS resulted in a significant loss of goblet cells that reflected the intensity of inflammation. The group treated with CypA displayed a loss of more than 60% of goblet cells (Figure 4C) and inflammation was still shown. However, goblet cells have been partially restored in the groups treated with CyA where around 70% of goblet cells were present throughout the tissue with localized inflammation (Figure 4D).

Similarly, the group treated with DSS and CypA-CyA complex, as shown in Figure 4E, showed a hyperactivity and inflammation with 50% loss of goblet cells. When probiotics was added to the complex, a mild progress was noticed to goblet cells and inflammation was still persistent (Figure 4F). On the other hand, when probiotic was given alone with DSS, 80% of goblet cells were retained and the architecture almost got back to normal (Figure 4G). Thus, the group given DSS + probiotics alone has clearly improved the colon's goblet cells and architecture.

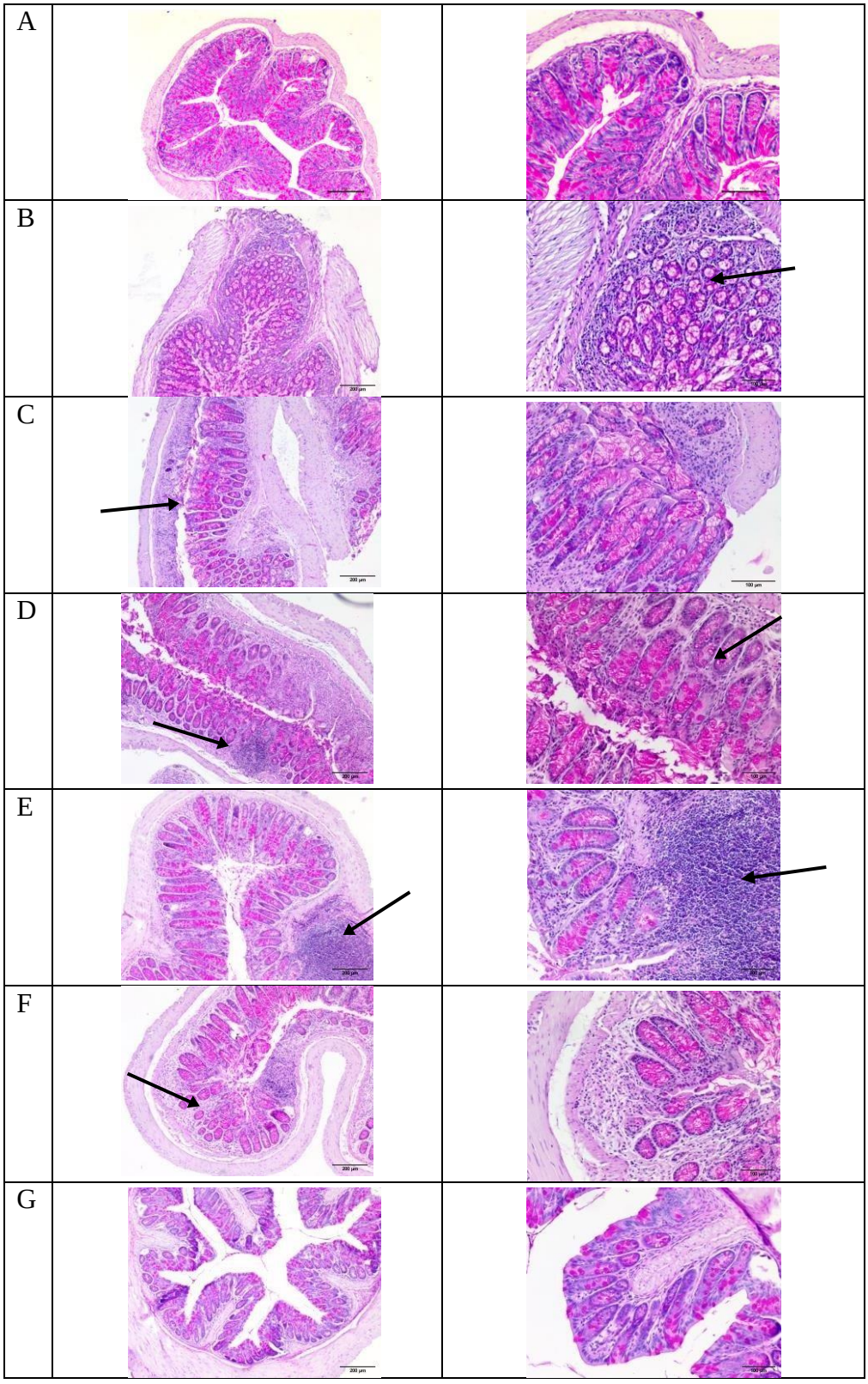

Figure 4. The representative photographs of wild type mice colon stained with Periodic acid-Schiff staining 40x magnification, scale bar 200 micron (left column) and 100x, scale bar 100 micron (right column). Arrows indicate reaction sites reaction.

As shown in Figure 5, in the Syndecan-1 knock out mice, the negative control group shows a normal tissue morphology where a vast amount of goblet cells resides throughout the mucosa layer (Figure 5A). However, a loss of 50%-80% of goblet cells is detected in the DSS-treated group (Figure 5B). Evidently, the effects of DSS produced a massive loss of goblet cells reflecting the severity of inflammation.

In contrast, in the group treated with (DSS + CypA), more than 75% of goblet cells have been present throughout the tissue, and about 25% have been only lost (Figure 5C). Similarly, the same effect has been revealed in the group treated with (DSS + CyA) where only 25% of goblet cells have been lost (Figure 5D).

On the other hand, the group treated with DSS plus (CypA-CyA) complex in (Figure 5E) presented a complete loss of goblet cells while the group treated with DSS + (CypA-CyA) complex + probiotics has retained about 25% of goblet cells (Figure 5F). In contrary, the treatment of (DSS + probiotics) only, in the absence of the complex, has evidently improved the colonic histopathological state in retaining 75% of goblet cells (Figure 5G) close to control.

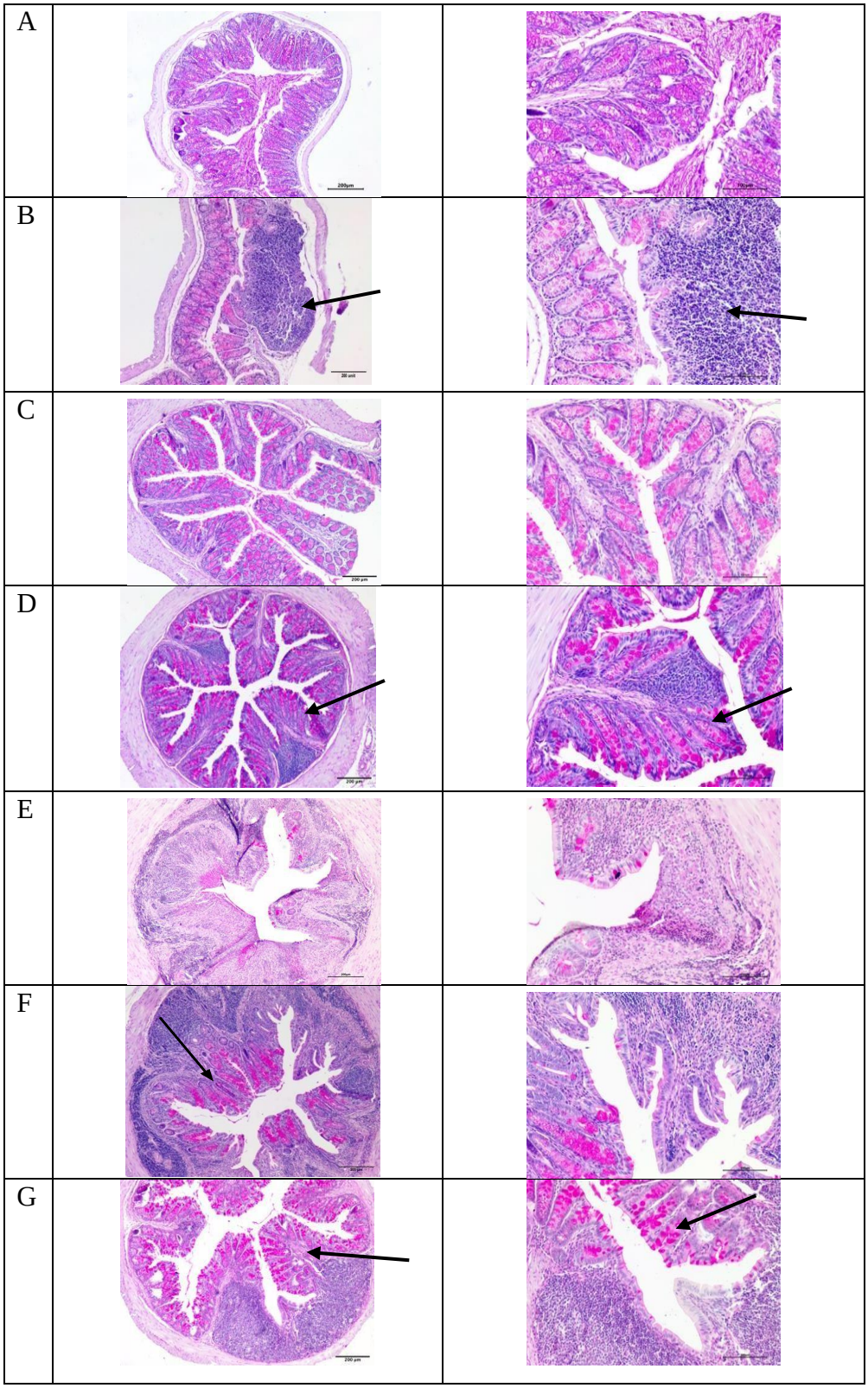

Figure 5. The representative photographs of Syndecan-1 knockout mice colon stained with Periodic acid Schiff staining 40x magnification, scale bar 200 micron (left column) and 100x, scale bar 100 micron (right column). Arrows indicate reaction sites. A shows normal presence of goblet cells throughout the epithelial lining in the normal no DSS treated control group1; Group 2 lost up to 80% of goblet cells (5B) while group 3 and group 4 CypA and CyA regained up to 75% (5C and D, respectively). On the other hand, the CypA-CyA complex in figure 5E showed almost complete loss of goblet cells. Treatment with probiotics and the CypA-CyA complex led to retainment of about 25% (5F) while probiotics alone (5G) retained about 75% of goblet cells.

Table 5: Comparative assessment of histological alterations in the colon of both strains of experimental mice

	Strain	
	Syndecan-1 null mice	Balb/C mice
Group 1 no DSS	Normal architecture, normal crypts, Continuous epithelial lining, normal distribution of goblet cells and elongated crypts- no inflammatory infiltrate	Normal architecture, normal crypts, Continuous epithelial lining, normal distribution of goblet cells and elongated crypts- no inflammatory infiltrate
Group 2 DSS only	Epithelial loss, loss of crypts, cryptitis, massive infiltration of inflammatory cells and invasion of the mucosa and submucosa and more edema between mucosa and submucosa, less decresed goblet cells	Epithelial loss, loss of crypts, cryptitis, infiltration of inflammatory cells, more decreased goblet cells
Group 3 DSS + Cyclophilin	Mild inflammation- 1/3 damaged crypts- low edema between mucosa and sub mucosa, same effect on goblet cells (decreased)	High inflammatory infiltrate- disorganized crypts and partial loss of epithelial lining, same effect on goblet cells (decreased)
Group 4 DSS + Cyclosporine	Limited infiltration of inflammatory cells- partial restoration of the epithelial lining, mucosal architecture and crypts integrity, more decreased goblet cells	Limited infiltration of inflammatory cells- Partial effect on epithelial lining- restoration of mucosal architecture and crypts integrity, less decreased goblet cells
Group 5 DSS + CypA + CyA	2/3 of the crypts are lost, intensive infiltration in mucosa and submucosa, epithelia loss in most of the places, more decreased goblet cells	Moderate restoration of the majority of mucosal architecture and crypts, less decreased goblet cells
Group 6 DSS + CypA+CyA + probiotics	Less effective than in wild type mice- Crypts improved, partial relief- moderate inflammation	Mild inflammation- restoration of the majority of mucosal architecture and crypts.
Group 7 DSS + probiotics	Some improvement, minimal restoration of mucosal architecture, moderate infiltration, less improvement than in wild type, same effect on goblet cells (restored)	Limited infiltration- Mild to no inflammation in some areas, restoration of the majority of the mucosal architecture and epithelial lining-Very effective- high Improvement, same effect on goblet cells (restored)

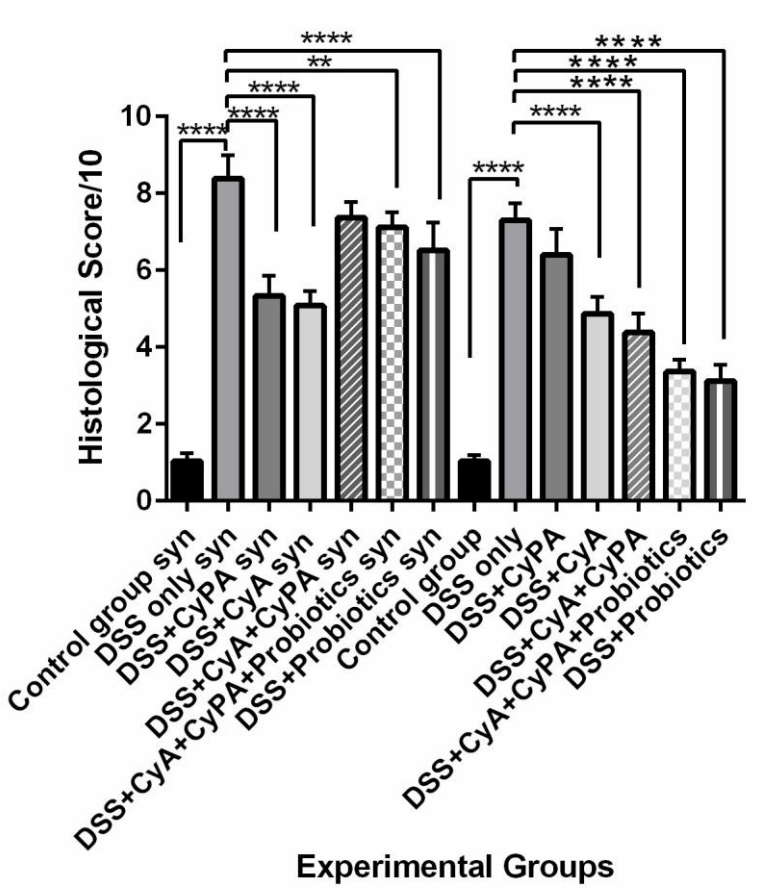

Figure 6. Histological alterations score. Effect of Cyclophilin A, Cyclosporine A and probiotics on colon histology in both strains. In Syndecan -1 null mice: the negative control group (no DSS) shows a score of (1.04/10), DSS group shows a score of (8.38/10). DSS+CyPA and DSS+CyA groups have the same histological score around (5/10). DSS+(CyPA-CyA) complex group shows a high score of (7.36/10). DSS+(CyPA-CyA) complex+probiotics group shows a score of (7.12/10) and DSS+probiotics group shows a score of (6.52/10). On the other hand, in bulb/c mice: the negative control group (no DSS) shows a score of (1.03/10), DSS group shows a score of (7.3/10). DSS+CyPA group shows a score of (6.4/10) and DSS+CyA group shows a score of (4.8/10). DSS+(CyPA-CyA) complex group shows a score of (4.36/10). DSS+(CyPA-CyA) complex+probiotics group shows a score of (3.36/10) and DSS+probiotics group shows a score of (3.12/10). Statistical significance is determined by one-way ANOVA. P-value < 0.05 is considered significant and is indicated by (*), P < 0.01 is indicated by (**), P < 0.001 is indicated by (***) and P < 0.0001 is indicated by (****). Data is expressed as Mean ±SEM (n=8).

3. Cyclophilin A, Cyclosporine A and probiotics modulate the expression of inflammatory some molecular markers: IL-6, CD147, CD3, Beta-1 integrins and pAkt – **Figure .**

Figure 7. Representative western blot bands for the expressions of IL-6, CD147, CD3, P-AKT and actin- in Balb/c mice (left column) and in Syndecan-1 mice (right column).

3.1 IL6

IL-6 is one of the well characterized proinflammatory cytokines. It is implicated in several signaling mechanisms and strongly involved in the pathogenesis of human and experimental IBD's. It can activate multiple pathways including the p13k-Akt pathway. In our experiments, IL-6 expression was assessed by western blot.

DSS administration increased significantly the expression of IL-6, evaluated by western blot, in both strains of mice compared to normal non-DSS treated controls; the ratio in the Syndecan-1 KO was 3.12 times and in the wild type 4.27 times. As a result of cyclophilin treatment this expression was inhibited compared to the DSS alone group 2 to significant extents with ratios of 0.40 and 0.44 in Syndecan-1 KO and wild type, respectively (Figure 8).

Similarly, cyclosporine A showed a similar pattern of inhibition by ratios of 0.45 and 0.56 in Syndecan-1 KO and wild type, respectively. However, with respect

to the combination CypA-CyA, the inhibitory effect did not add up, in particular with the Syndecan-1 KO where the inhibition of expression was decreased to a 0.62 ratio and more so, 0.47, in the wild type (Figure 8).

On the other hand, treating with probiotics alone the inhibition of IL-6 expression was very significant compared to all the groups with 0.4 expression compared to the DSS only treatment in Syndecan-1 KO and more so, 0.31 in the wild type (Figure 8).

Probiotics seems to be more efficient than all treatments in lowering IL-6 expression; however, when used with the CypA-CyA complex its inhibitory effect of IL6 is much less. The Syndecan-1 KO responded relatively more to cyclosporine and cyclophiline when used separately, which is not the case with the CypA-CyA complex whereby the inhibitory effect for IL-6 expression was significantly less. On the other hand, the wild type responded slightly different by keeping the inhibition of IL-6 expression similar to CypA and less than CyA (Figure 8).

In conclusion, comparing the 2 strains, the CypA-CyA complex was less effective in decreasing the level of IL-6 expression in the Syndecan-1 KO with a ratio of 0.62 compared to the wild type 0.47. The wild type IL-6 expression was less affected by the complex. Furthermore, the probiotics had less effect in reducing IL-6 expression in Syndecan-1 KO compared to wild type both in combination with the CypA-CyA or separately.

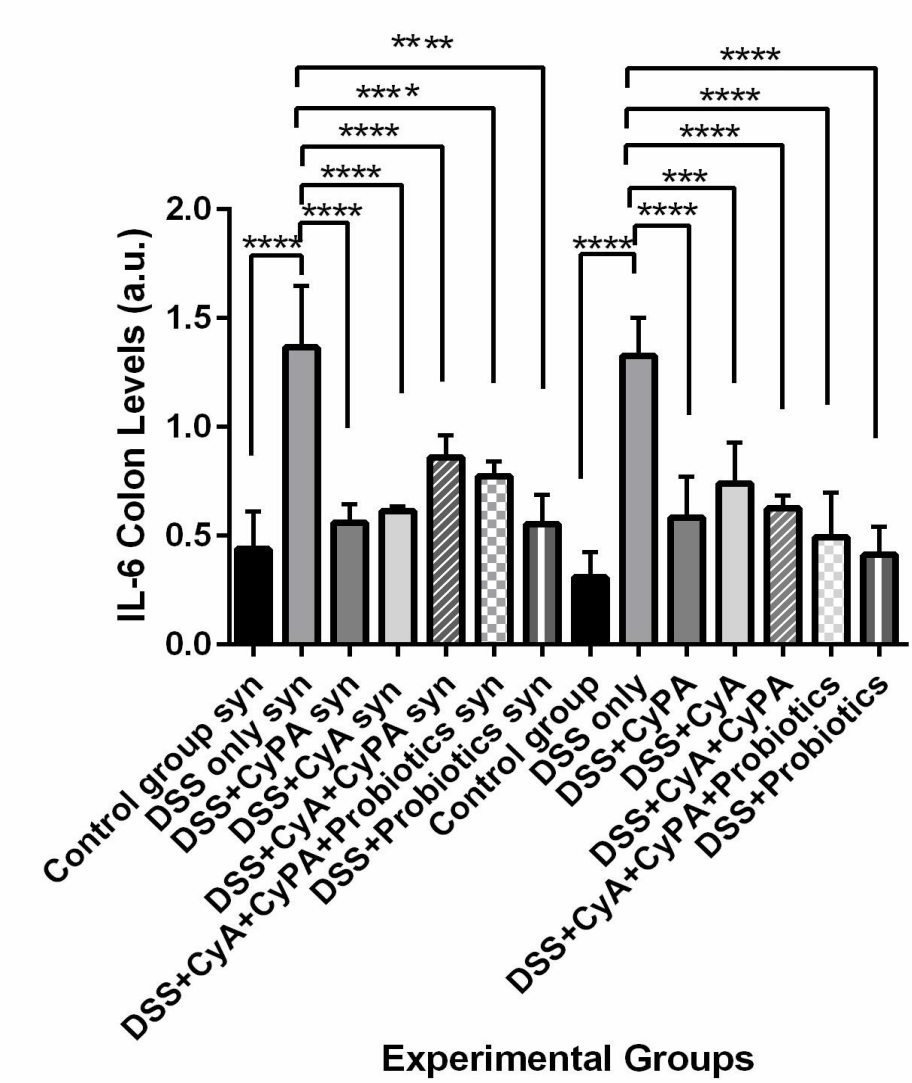

Figure 8. Variation of IL-6 levels evaluated by western blot technique from colon extraction of the different experimental groups. P-value < 0.05 is considered significant and is indicated by (*), P < 0.01 is indicated by (**), P < 0.001 is indicated by (***) and P < 0.0001 is indicated by (****)

3.2 CD 147

It has been proven that CD147 is implicated in a plethora of cellular processes such as signal transmission, cell adhesion, neural functions, and reproduction. It plays an essential role in inflammation and immune responses. Studies have demonstrated that an elevated expression of CD147 has been detected in the intestinal mucosa of IBD patients showing that it is a key proinflammatory

biomarker for IBD [530]. In our experiments, CD147 expression was assessed by western blot.

DSS treatment or induction of IBD caused significant increases in the expression of CD 147 in both strains 1.34 and 1.32 in KO and wild type, respectively. However, in Syndecan-1 KO mice the relative ratio to the normal control was significantly higher 1.34/0.24=5.6 times compared to 1.32/0.8=1.65 times than the wild type (Figure 9).

The CypA decreases the expression of CD147 significantly compared to the DSS group 0.44 ratio in KO mice compared to 0.63 ratio in the wild type, and similarly behaved cyclosporine 0.44 compared to 0.66 ratios in the Syndecan-1 KO and wild type, respectively (Figure 9).

However, when the complex CypA-CyA was the treatment, the expression of CD147 increased in Syndecan-1 KO compared to each drug separately to reach an expression ratio of 0.7 compared to the DSS alone while the wild type ratio remained about 0.5 (Figure 9).

The use of probiotics alone maintained a lower expression of CD147 similar in both strains 0.43 Syndecan-1 KO and 0.40 in the wild type. However, adding probiotics to the CypA-CyA combination improved the inhibitory effect on CD147 expression in both strains compared to the CypA-CyA combination without probiotics with ratios of 51 in KO mice and 45 in wild type; relatively more effect of probiotics in the KO mice in lowering the CD147 expression in the combination (Figure 9).

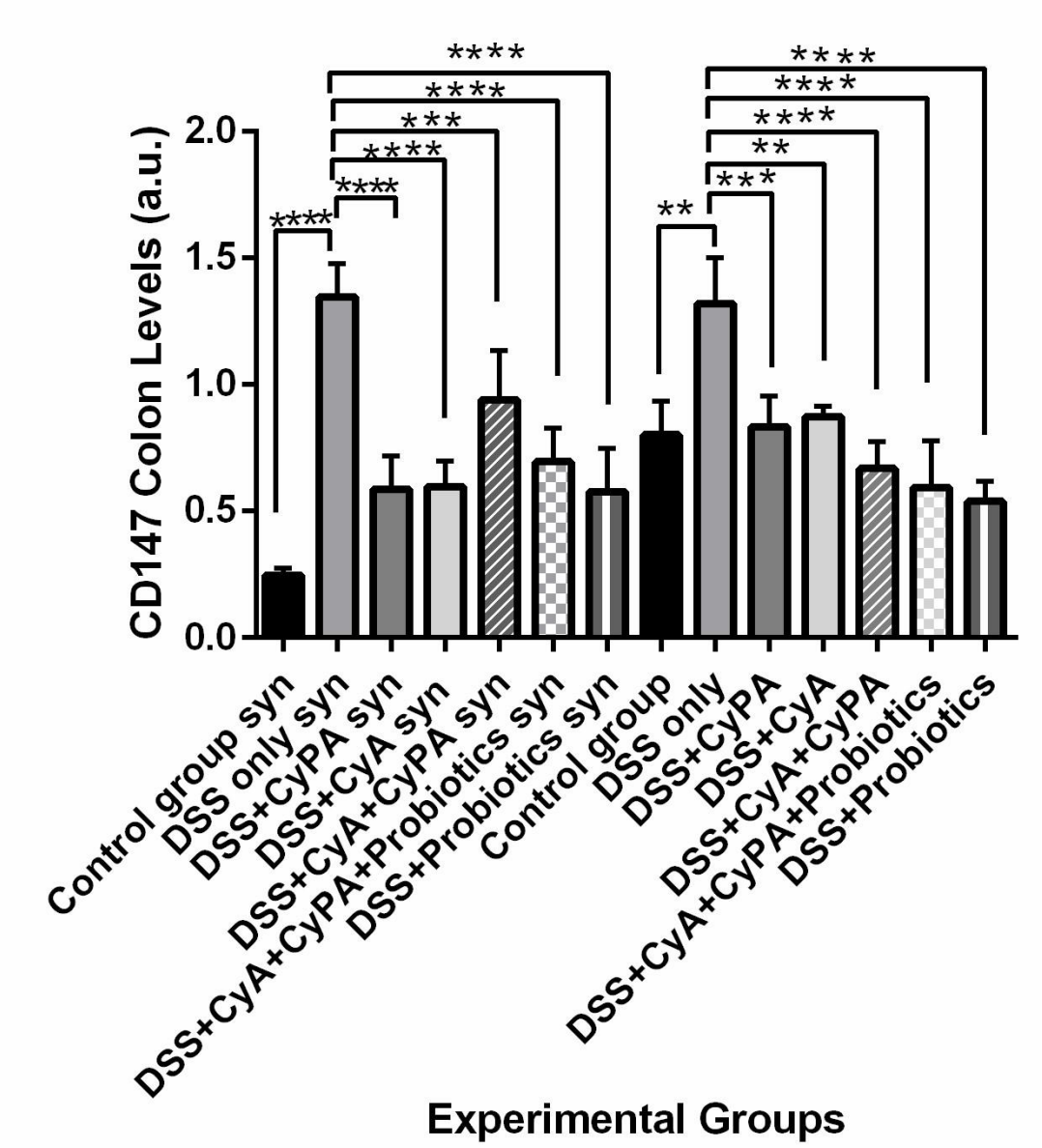

Figure 9. Variation of CD147 levels, evaluated by western blot technique, from colon extraction of the different experimental groups. P-value < 0.05 is considered significant and is indicated by (*), P < 0.01 is indicated by (**), P < 0.001 is indicated by (***) and P < 0.0001 is indicated by (****).

3.3 CD3

The CD3 antigen is a surface structure associated with the T-cell receptor (TCR) to form a complex involved in antigen recognition and signal transduction. The CD3–T cell receptor (TCR) complex plays a central role in the T-cell-mediated immunoresponse as it is involved in the recognition of antigens and subsequent signal transduction and activation of immunocompetent T lymphocytes. It is proven

that CD3 is an inflammatory marker that is upregulated during inflammatory diseases, including “IBD” [573]. In our study CD3 was assessed by Western Blot.

CD3 expression was significantly elevated in both strains of mice as a result of induction of IBD with DSS to reach a ratio of 7.45 in the KO mice and 4.25 in the wild type, respectively, compared to the control non-DSS treated mice; a severe inflammatory reaction in both strains (Figure 10).

Treatment with cyclophilin A decreased the CD3 expression compared to the DSS group by a ratio of 0.53 in KO mice and 0.44 in wild type. Similarly, CyA decreased significantly CD3 expression, as expected, to a ratio 0.42 in KO mice and less so in the wild type 0.62, compared to the DSS alone group, leading to the more significant decrease of CD3 expression in the KO mice (Figure 10).

The use of the CypA-CyA combination did not have any added value for the suppression of CD3 expression, in the KO mice it increased the expression compared to either CypA or CyA alone with a ratio of 0.64 while in the wild type the ratio was 0.51. Adding probiotics to the CypA-CyA combination lowered the CD3 expression compared to the combination without probiotics to a ratio of 0.47 on KO mice and much more, 0.26, in wild type. Again, the effect of probiotics in the combination differed between the two strains when added to the combination; it was more effective in suppressing CD3 in the wild type. On the other hand, both strains responded relatively well to the probiotics treatment alone and decreased the expression of CD3 to significant levels compared to the DSS alone group, the ratios were close with a margin for the KO mice with a ratio of 0.23 versus 0.31 for the wild type (Figure 10).

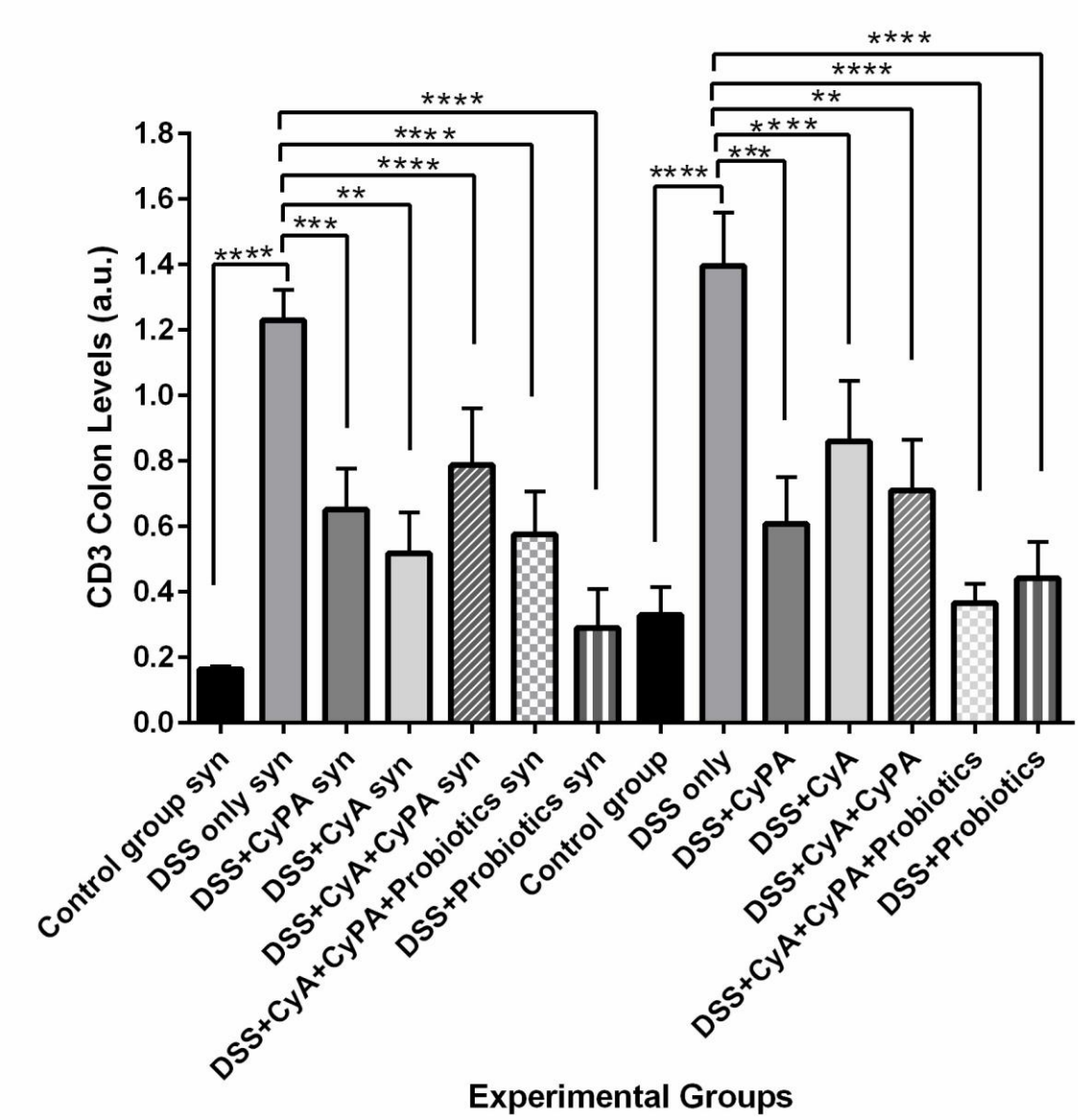

Figure 10. Variation of CD3 levels, evaluated by western blot technique, from colon extraction of the different experimental groups. P-value < 0.05 is considered significant and is indicated by (*), P < 0.01 is indicated by (**), P < 0.001 is indicated by (***) and P < 0.0001 is indicated by (****).

3.4 ß 1 integrin

Evidence confirms that the pathogenesis of IBD includes an increase in infiltrating gut-homing leukocytes, a process known to be tightly regulated by a stepwise process involving multiple integrins, in particular, Beta-1 integrins.

Beta-1 integrin is highly expressed in IBD, in particular, in the lamina propria lymphocytes (LPLs) and neutrophils as well as other inflammatory cells, in

the inflamed intestinal tissues and the gut associated lymphoid tissue (GALT). Patients with active IBD have increased expression of activated Beta-1 integrin on more than 80% of their CD4+ LPL's [586, 587].

In our experiments, Beta-1 expression was assessed by immunofluorescence using anti Beta-1 antibodies. Screening of the histological sections for active Beta-1 expression in the various groups of both strains of mice, the wild type as well as the Syndecan-1 knock-out, showed the following (Figure 11).

Beta-1 integrin was expressed to similar extents in the normal control groups one in both strains 10.78 in Syndecan-1 knock out and 11.27 in the wild type. In addition, as expected, significant increases in Beta-1 integrin happened as a result of DSS stimulation without any treatments in both strains, almost twice as much in the wild type, 50.23 with a ratio of 4.45 compared to control, and 29.63 with a ratio of 2.74 in the Syndecan-1 knock out mice. Very significant increases in both strains. Treatment with cyclophilin A significantly decreased the Beta-1 integrin expression, again in both strains, but more so in the wild type with a ratio of 0.36 compared to 0.61 in the knock out, that is almost 25% less expression in the knock out mice (Figure 11).

In addition, cyclosporine A treatment also decreased more than cyclophilin the expression of Beta-1 integrin in comparison with groups 2 of DSS alone. The expression in the knock out was 14.78 with a ratio of 0.49 and significantly less in the wild type, 9.64 with a ratio of 0.19. The suppression of expression was very much more significant in the wild type compared to the knock out mice (Figure 11).

However, treatment with the combination of CypA-CyA did not add to the reduction in expression produced by CyA alone in both strains; the ratios were 0.46 for the knock out and 0.19 for the wild type. On the other hand, adding probiotics to the CypA-CyA combination, led to a high expression of Beta-1 integrin in both strains, rather than reduction, way beyond the combination of CypA-CyA or any drug alone CypA-CyA. The expression in the wild type was reduced more 13/50.2 with a ratio of 0.26 compared to a significantly much less suppression effect compared to group 2 (DSS alone) 20.9/29.6 with ratio of 0.7. Probiotics did not have any added value to reduce the expression of Beta-1 integrin when included in the treatment with the combination. It was just the opposite, an increase way beyond either drug alone or even the combination and much more so in the knock out mice (Figure 11).

On the other hand, probiotics alone did reduce significantly the expression of Beta-1 integrin in both strains but less so in the knock out mice (0.6) compared to the wild type (0.17) ratios (Figure 11).

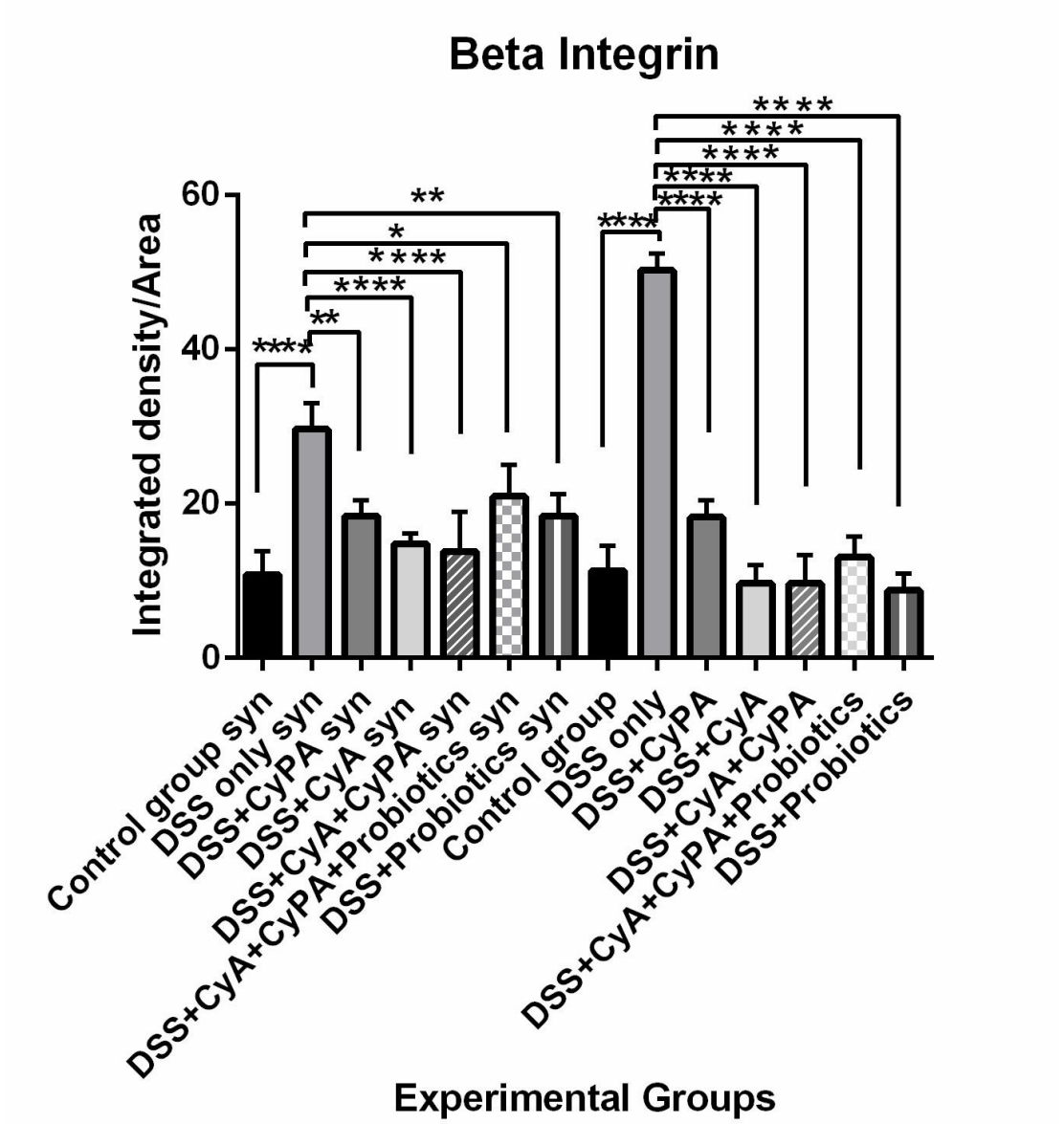

Figure 11. Variation of ß 1 integrin levels, assessed by immunofluorescence technique, of the different experimental groups. P-value < 0.05 is considered significant and is indicated by (*), P < 0.01 is indicated by (**), P < 0.001 is indicated by (***) and P < 0.0001 is indicated by (****).

3.5 pAKT

The activated phosphorylated for the Akt (pAkt) modulates the function of many substrates involved in the control of differentiation of regulation of cell growth and survival by inhibiting other apoptotic processes. It is also involved in

autoimmune disorders. The p13k/Akt signaling pathway is upregulated in mice IBD [583, 585]. In our study pAkt was assessed by Western Blot.

As shown in Figure 12 the pAkt in response to the DSS induction of IBD was upregulated in both strains of mice to various levels favoring the KO with 1.91 and 1.46 in the wild type with ratios of 1.74 in the KO and 1.63 in the wild type compared to the control normal group. In the Syndecan-1 KO mice, the treatment with cyclophilin reduced the expression of pAkt to the level of normal control and so did cyclosporine with ratios of 0.57 and 0.58, respectively. This decrease in pAkt expression was further reduced to a ratio of 0.39 when the combination was used. However, adding probiotics to the combination brought back a similar effect to either compound alone with a ratio of 0.59 (Figure 12). On the other hand, probiotics alone behaved very much like the combination with a ratio of 0.39.

In the wild type the expression of pAkt was significantly lower across all groups to a great extent. The DSS alone group had an expression ratio of 1.46 while the cyclophilin A and cyclosporine had 0.5 and 0.68 and when combined they had and added effect, like in the KO mice, leading to a ratio 0.32 close to that of the KO ratio of 0.39. Similarly, adding probiotics to the combination of CypA-CyA led to a ratio of 0.5 close to the KO mice of 0.59. Again, The KO and wild type mice behaved similarly with respect to pAkt expression when the treatment was probiotics alone reaching ratios of 0.39 for KO mice and 0.45 for wild type (Figure 12).

In this case the combination as well as the probiotics decreased pAkt expression significantly in both strains to similar extents.

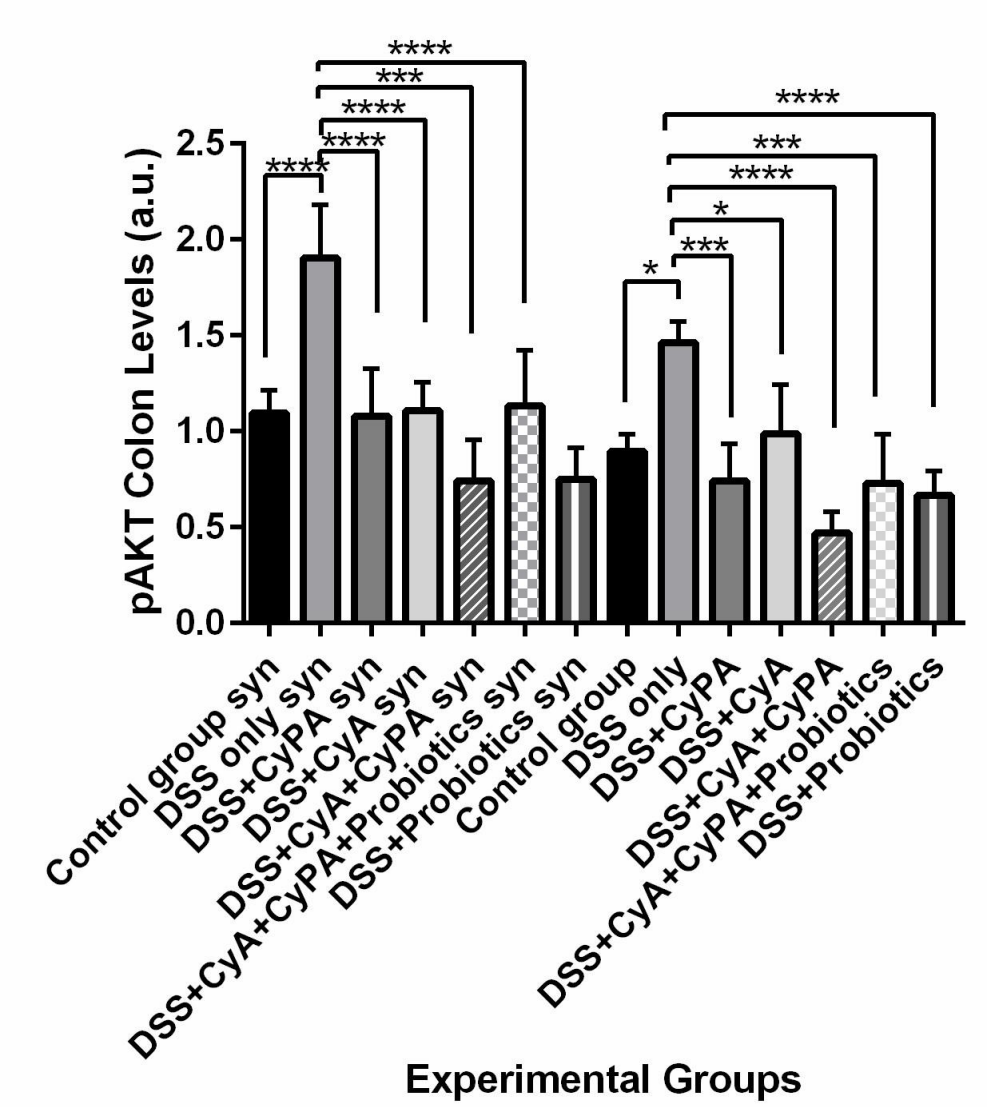

Figure 12. Variation of pAKT levels, assessed by western blot technique, from colon extraction of the different experimental groups. P-value < 0.05 is considered significant and is indicated by (*), P < 0.01 is indicated by (**), P < 0.001 is indicated by (***) and P < 0.0001 is indicated by (****).

Table 6. Effect of Cyclosporine or Cyclophilin on the various parameters in both strains

	Syndican -1/KD	**Wild type**
Clinical signs and symptoms	More severe	Less severe
Histological alterations	More severe	Less severe
IL-6	More decreased	Less decreased
C147	More decreased	Less decreased
CD3	More decreased	Less decreased
β1 Integrin	Less decreased	More decrease
pAKT	Less decreased	More decrease

Table 7: Effect of Probiotics on the CYA-CYPA combination

	Syndican -1/KD	**Wild type**
Clinical signs and symptoms	Slightly more	Same or less
Histological alterations	More severe	Less severe
IL-6	Less effect/ More IL-6	More effect/ Less IL-6
C147	Less effect/ More C147	More effect/ Less C147
CD3	Less effect/ More CD3	More effect/ Less CD3
β1 Integrin	Less effect/ More β1 Integrin	More effect/ Less β1 Integrin
pAKT	Less effect/ More pAKT	More effect/ Less pAKT

CHAPTER V
DISCUSSION

Accumulating evidence describe IBD's as chronic autoimmune inflammatory gastrointestinal disorders. They result from a dysregulated immune response with inflammation, genetic susceptibility and dysbiosis being the underlying mechanisms.

Treatments to these disorders, which constitute a group of diseases Chron's, ulcerative colitis and others, have evolved over the years to reach recently targeted therapy, genomic medicine and immunotherapy, as well as probiotics.

This study focused on the role of Syndecan-1 in the pathogenesis and management of IBD, using a Syndecan-1 knock out strain of mice in comparison to a wild type Balb/c strain, in combination with the immunosuppressant cyclosporine A. Cyclophilin A, which has multifunctional properties in cell physiology and pathological conditions, particularly when over expressed, contributes to inflammatory diseases [477] by stimulating proinflammatory signals involving communication with the membrane receptor CD147. Besides its role as a ligand for CD147, CypA serves as a receptor for CyA, thus creating a complex responsible for immunoregulation. In this context, this study assessed multiple inflammatory markers like IL-6, CD147, Beta-1 integrins, CD3 as well as pAkt, a pathway known to be involved in the management of IBD. Adding probiotics to the management protocol of IBD is based on accumulating evidence of their efficiency and their beneficial effects in treating IBD's [8, 9].

Data from this study showed that the absence of Syndecan-1 in knock out mice led to an exacerbating inflammatory reaction more intense and more lethal to the animals at doses that are tolerated by the wild type mice. In a pilot study at the start of the experimental work, a kinetic experiment of dose response to select the optimal dose was performed.

Results showed that the absence of Syndecan-1 made the mice more sensitive to DSS stimulation whereby, almost half of the animals died within 4 days. On the other hand, the wild type mice were more resistant and could stand higher doses than 2%, the daily dose used for the Syndecan-1 knock out mice. Such data speak for the importance of Syndecan-1 and its role in the mechanisms involved in IBD. Besides its modulation of different proteolytic activities [397], Syndecan-1 plays a crucial role in a plethora of biological processes including cell proliferation, differentiation and redifferentiation [397] as well as in improving wound healing and epithelium integrity. Consequently, its absence will delay healing, repair and maintenance of the mucosa barrier. These functions are achieved through its role in integrin activity and migration as well as enhancing the motility of macrophages. Syndecan-1 is also associated with an anti-inflammatory M2 macrophage polarization [403] involved in maintaining the function of mucosa barrier and restoration of tight junctions to maintain epithelial cell integrity through activating stat 3. The presence of Syndecan-1 could also inhibit the secretion of proinflammatory cytokines due to its suppressed ectodomain shedding thus causing amelioration of intestinal inflammation and neutrophil transmigration [438]. On the other hand, the loss of Syndecan contributes to epithelial permeability and defect in the barrier leading to microbiota access to lamina propria and causing more

chronic and persistent inflammatory reaction and accumulation of inflammatory cells. All these reactions were encountered in this study and documented through histological studies as well the increase in the daily activity index (DAI) index as well as the symptoms and signs; all reflected more severity of the induced IBD in the knock out mice compared to the wild type.

Most available treatment protocols for IBD aimed to maintain patients in remission, ameliorate symptoms, reduce severity and prevent surgeries [440]. Immunoregulators, including the classical immunosuppressive drug cyclosporine A (CyA), have used in IBD therapy, in particular in ulcerative colitis [450-453]. CyA has a pivotal role in ameliorating chronic inflammatory diseases [459]. It downregulates the activation of T-lymphocytes by blocking the production of IL-2, inhibits the production of proinflammatory cytokines and stimulates apoptosis [450-453]. Its mechanism of action starts when it binds to CypA, an intracellular binding protein for CyA, forming a CypCypA complex, thus affording an anti-inflammatory activity [466].

In this study, cyclosporine A, inhibited intestinal inflammation to a limited extent. The symptoms and signs improved, the histological alterations improved to significant extents in both strains.

In addition, fistula closure in the sigmoid colon was comparable in both strains. It also inhibited intestinal inflammation and reduced colitis and colonic destruction significantly in both strains. CyA also decreased inflammatory cells infiltration in mucosa and submucosa and the GALT as evidenced through the histological analysis. CyA in the knock out mice caused more sever inhibition of IL-6 compared to the wild type as well as more suppression n the expression of CD147 and CD3

and less effect on Beta-1 integrins. The pAkt which was highly expressed as a result of DSS decreased back to control levels as a result of CyA in both strains to variable degrees with an edge to the knock out mice.

Therefore, in the absence of Syndecan-1, CyA was very effective in controlling the symptoms, improving and limiting histological alteration, and decreased proinflammatory cytokines expressions. However, such effects were more obvious in the knock out mice.

Data from this study showed that the CypA treatment in the Syndecan-1 knock out mice showed a decrease in the inflammation caused by the DSS, contrary to what is being reported in the literature, upregulation of CypA in patients with inflammatory disorders [497, 513-516]. Such an anti-inflammatory effect of CypA was more in the knock out mice compared to the wild type at the histological level with reduced alterations in mucosa, crypts and inflammatory cells infiltrate as well as goblet cells. The same effect was depicted at the level of proinflammatory markers with a similar inhibition of IL-6 in both strains, 0.4 and 0.44 ratios compared to DSS alone group in the knock out and wild type, respectively. However, Beta-1 integrins and CD3 were more inhibited in the wild type while CD147 was less expressed in the wild type compared to the knock out mice. In general, CypA downregulated inflammation across the board but more so in the knock out mice and the pAkt which was highly elevated in the DSS alone, in particular, in knock out mice was back to normal control levels in both strains of mice. Therefore, CypA which is the primary cytosolic binding protein of CyA, was provided intraperitoneally, thus creating a high concentration of extracellular CypA

which can potentially bind to and block CD147 instead of stimulating their chemotactic activity.

The pAkt by going back to its normal control levels, that is deactivated, showed that CypA could interplay with the Akt pathway and somehow downregulates its activity in both strains. Such an effect is expressed in both strains and slightly more in the knock out mice with respect to better tissue repair, more IL-6 suppression, more CD147 suppression and less Beta-1 integrins expression which favors the better tissue repair.

Concerning the use of the combination of CyA+ CypA with the expectations of having an added anti-inflammatory effect in IBD, the results were not in favor of such a hypothesis. Actually, the use of the combination led to a moderate restoration of intestinal mucosa with crypt and goblet cells in the wild type. On the other hand, there was an exacerbation in the absence of Syndecan-1 in the biopsies from the sigmoid colon of knock out mice with intensive inflammatory cellular infiltration and loss of epithelium and goblet cells. The expression of IL-6 increased significantly compared to either drug alone in the knock out mice, the CD147, Beta-1 integrins and the CD3 behaved the same, all pointing to an increase in the inflammatory process.

Moreover, the pAkt followed the same pattern of having activated expression much more significantly in the absence of Syndecan-1.

In brief, the data of this study showed no added effect of the CypA+ CyA combination in reducing further the inflammatory reaction in the intestines. On the contrary, there was an exacerbation in the knock out mice versus a partial added

effect in the wild type as expressed in the lowest pAkt, lowest Beta-1 integrins levels, lower CD147 levels and lower IL-6 levels.

These findings are not in line with reports from the literature. Such an aggravation of inflammation in this combination group could probably be ascribed to the excess CypA administered along with the cyclosporine, thus forming a CypA+ CyA complex that could probably stimulate significant relative increase in Beta-1 integrins, CD3, CD147 and even IL-6 more in the absence of Syndecan-1. Such changes in the knock out group activated pAkt and probably other signaling pathways leading to such exacerbation of the disease. Actually, physiologically these inflammatory markers can induce the activation of multiple pathways; the Janus kinase (JAK) signal transducer and activator of transcription 3 (STAT 3) pathway, the Src homology 2 (SH2)- containing protein tyrosine phosphatase-2 (SHP-2), the extracellular signal-regulated kinase (ERK) pathway, and the phosphoinositide 3-kinase (PI3K)-Akt pathway. For example, IL-6 influences the differentiation of myeloid lineages, involving dendritic cells and macrophages which are highly involved in this pathogenesis via STAT 3 activation (463R). Il-6 can also activate PI3K-Akt. When Akt is fully activated in its phosphorylated form (pAkt), it moves to the cytoplasm and nucleus to phosphorylate several downstream targets and subsequently regulate cellular functions. It has been proven that pAkt is implicated in IBD pathogenesis where a significant increase in its expression has been reported in DSS model of colitis (445R) and that is the case in this study. In addition, pAkt signaling has a pivotal role in the interaction between tissue infiltration of macrophages, mast cells and epithelial cells in colitis-induced cancer. Such activities lead to crypt architectural disturbances found in colitis. Such a

signaling pathway could be better activated among others in the absence of Syndecan-1 as evidenced from the data of this study.

In addition, several studies have previously shown that activated Akt affects T cell activation and survival by restraining apoptotic processes. Our data on pAkt expression in the DSS treated group of both strains are consistent with previous studies demonstrating that pAkt inhibited T cells apoptosis and contributed to a more activation of T cells leading to an increase in CD3 expression. Data from the knock out mice are in agreement with such reports. Off note, CypA has been shown to be involved in activating Akt and NF-KB signaling pathways thus aggravating the status of inflammation. In this study, there was maybe excess CypA or a saturation status, which with the CyA could form a complex that is not probably immunosuppressing to the expected level. More explorations are needed concerning the mechanisms implicated in such unexpected response of exacerbation by the combination.

Adding probiotics to the combination in both strains produced a differential in the expression of the inflammation markers between the Syndecan-1 knock out and the wild type. The DAI in the knock out mice slightly less in the combination, there was no significant improvement in the index. The inflammation in the colon showed more significant improvement in the histology and less alterations in the wild type, in comparison to slight overall improvement with the knock out mice. The same pattern applied to IL-6, which had more significant suppression in the wild type, to CD147, to CD3, to Beta-1 integrins and pAkt.

In brief, the presence of probiotics with the combination improved the health status of the animals, more so in the wild type than in the Syndecan-1 knock

out mice. Despite the inflammation in the combination with probiotics, the clinical signs and symptoms were improved which reflects a better intestinal barrier preserved by the presence of probiotics. More investigations are needed to unveil the mechanism of action of the complex in the presence of probiotics; are they working with same or a different mechanism?

On the other hand, the addition of probiotics alone, in the absence of (CypA-CyA) complex revealed a marked reduction in inflammation in both strains but significantly more in the knock out mice. Histology of the sigmoid colon showed almost normal preserved architecture in 75% of the section, yet very active Lymphocytes aggregates existed with a relatively normal epithelial barrier. Regarding the molecular parameters, the levels of proinflammatory markers were notably reduced compared to that in DSS+(CypA-CyA) complex and DSS+(CypA-CyA) complex plus probiotics. The values of IL-6, CD147, CD3, Beta-1 integrins, and pAKT expressions in (DSS+ probiotics) group have significantly decreased compared to those in DSS+(CypA-CyA) complex group and more so in the wild type. In brief, probiotics alone are more efficient than both drugs in reducing the inflammation in the colon. This result is in line with previous studies which have evidently shown that probiotics stimulate the differentiation of T-helper 1 cells, boost antibody production, promote the activity of both natural killer cells and phagocytic cells, and increase T-cell apoptosis by inhibiting the transcription of NF-KB. In addition, they have a pivotal role in increasing the production of anti-inflammatory cytokines while decreasing that of proinflammatory cytokines [532, 593-595]. Moreover, probiotics prevent apoptosis of intestinal epithelial cells and stimulate the production of proteins that are essential components of tight junctions,

thus decreasing the paracellular permeability and restoring the barrier function, in agreement with our results [596-599]. On the other hand, probiotics produce bacteriocins, thus creating an acidic medium detrimental to pathogenic bacteria, yet favorable to the growth of beneficial microorganisms such as lactobacilli and bifidobacteria [549, 600-602]. Furthermore, in a DSS-induced model of colitis, it has been shown that the supplementation with a mixture containing Bifidobacterium, Lactobacillus acidophilus, and Enterococcus has consequently contributed to a reduction in total T-cells and increase in the number of Treg cells in the colonic tissue and blood, in addition to enhancing the function of tight junctions [556]. In this study, the results of the group treated with DSS+(CypA-CyA) complex+ probiotics demonstrated a slight reduction in CD3 expression compared to DSS+(CyA-CypA) group while a sharp increase has been observed in the (DSS+ probiotics) group where (CypA-CyA) complex is absent. However, the presence of probiotics in DSS+(CypA-CyA) complex+ probiotics group has been shown to partially decrease the effectiveness of probiotics by immunosuppressing T-cells more in Syndecan-1 knock out mice compared to wild type. The significant reduction in the molecular markers (IL-6, CD147, Beta-1 integrins, CD3 and pAKT) fosters the same conclusion, that probiotics are less effective in the presence of (CypA-CyA) complex, in particular in the knock out mice. However, the mechanism underlying the action of CyA and CypA in presence of DSS and probiotics need further and deeper investigations. Would it be possible that the complex (CypA-CyA) could override the effect of probiotics? Or cyclophilin would then work as a proinflammatory agent?

On the other hand, the marked reduction in inflammation was clearly noticed in (DSS+ probiotics) only, supporting the fact that probiotics decrease the production of anti-inflammatory cytokines and have a significant role in relieving inflammation.

Altogether, our findings suggest a therapeutic role for cyclophilin “A” in DSSinduced sdc-1 deficient mice. The presence of distinct receptors for extracellular CypA (other than CD147 and sdc-1) on its target cell merits further exploration. In addition, the potent role of cyclosporine in IBD therapy has been confirmed in both strains as demonstrated by the marked reduction of inflammation taking place in (DSS+CyA) group. However, the exacerbation of inflammation in the group treated with the complex group (CypA-CyA) needs further investigation since it is occurring mostly in the Syndecan-1 knock out mice. Moreover, the effectiveness of probiotics has been clearly revealed when used alone in DSS-induced sdc-1 deficient mice as well as the Balb/c wild type. In contrast, this effectiveness has been partially inhibited in the presence of (CypACyA) complex. Further and deeper investigations need to be carried out to answer the question on how the combination decreases the effect of probiotics and what are the mechanisms involved?

CHAPTER VI
LIMITATIONS

Limitations of the study

- This study has its limitations with respect to the number of animals i.e. Syndecan1 knock out mice, which were difficult to breed and get good numbers of progenies. This limitation led decrease in the types of groups whereby a group of CyA with probiotics and a group of CypA with probiotics could have been added in order to have a more complete picture.
- There was also limitations in the budget to purchase and test for more inflammatory markers as well as TGFβ and TNFα among others, to explore more the various signaling pathways that are relevant and probably involved in the process.

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