Role of cytokine IL-15 in the homeostasis of the intestinal epithelium and inflammatory bowel diseases.

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Programme de Biologie Cellulaire

Master's thesis presented to the Faculty of Medicine and Health Sciences in view of obtaining a Master of Science (MSc.) in Cell Biology

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UNIVERSITÉ DE SHERBROOKE

Sherbrooke, Québec, Canada
August, 2019

RÉSUMÉ

Rôle de la cytokine IL-15 dans l'homéostasie de l'épithélium intestinal et les maladies inflammatoires de l'intestin.

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Mémoire présenté à la Faculté de Médecine en vue de l'obtention du grade de Maître en Sciences (MSc.) en Biologie Cellulaire

Les maladies inflammatoires intestinales (MII), telles que la colite ulcéreuse et la maladie de Crohn, sont caractérisées par une augmentation de la perméabilité intestinale causée par un dysfonctionnement de la barrière muqueuse intestinale, facteur déterminant de la pathogenèse des MII. Le système immunitaire est le principal responsable de ces lésions tissulaires, par l'action de multiples types de cellules ainsi que de médiateurs solubles, tels que le TNFα, qui sont actuellement des cibles thérapeutiques dans le traitement des MII. L'interleukine 15 (IL-15) est une cytokine pro-inflammatoire impliquée dans le maintien et l'activation de sous-groupes de lymphocytes cytotoxiques et de macrophages dans l'épithélium intestinal, en agissant par l'intermédiaire du récepteur trimérique, l'IL-15R. L'expression de l'IL-15 est augmentée chez les patients atteints de MII et dans les modèles murins d'inflammation intestinale. Cependant, le rôle exact de la signalisation de l'IL-15 dans l'homéostasie intestinale et les réponses inflammatoires, en particulier dans les cellules épithéliales intestinales (IEC), reste à déterminer. La fonction de l'IL-15 a été largement décrite dans les cellules immunitaires, mais ses fonctions dans d'autres types cellulaires sont moins bien connues. Le but de cette thèse est d'étudier le rôle de l'IL-15 dans l'homéostasie de l'épithélium intestinal et les maladies inflammatoires de l'intestin.

Nous avons testé l'influence de l'IL-15 sur l'homéostasie intestinale chez les souris C57BL/6 de type sauvage (WT) et déficientes en IL-15 (*Il15*$^{-/-}$). Après 7 jours, l'analyse macromorphologique de l'intestin grêle et du colon de souris nulles en *Il15* n'a révélé aucune anomalie spontanée par rapport aux souris WT. Pour déterminer l'impact de la signalisation de l'IL-15 sur la prolifération et la réparation intestinales sous un stress exogène, nous avons exposé des souris WT et *Il15*$^{-/-}$ au dextran sulfate de sodium (DSS), un irritant chimique qui perturbe la barrière épithéliale intestinale et induit la colite. Les souris déficientes en IL-15 ne présentaient pas une sensibilité diminuée à la colite aiguë induite par le DSS. De plus, nous avons constaté que le blocage de la signalisation de l'IL-15 dans la colite induite avec DSS par l'anticorps TM-β1, qui se lient à la chaîne β du récepteur partagé par les cytokines IL-2 et IL-15, empêche l'apparition globale de l'inflammation associée à la colite chez les souris *Il15ra*$^{-/-}$ mais pas chez les souris WT.

En utilisant des entéroïdes de cryptes provenant de souris WT et déficientes en *Il15*, cette étude démontre que l'IL-15 exogène induit l'augmentation du niveau d'ARNm du marqueur de cellules souches *Lgr5* dans les entéroïdes mutants, mais pas les autres marqueurs de lignées des cellules épithéliales testés, suggérant un rôle de l'IL-15 dans la prolifération et la régénération des cellules intestinales. Les organoïdes intestinaux stimulés par IFNγ réduisent de manière significative la prolifération basale et la viabilité *in vitro* dans les entéroïdes WT par rapport à ceux déficients en *Il15*. Fait intéressant, une différence réduite dans l'expression des gènes *Tnfa* et *Cxcl10* a été observée dans les entéroïdes déficients en *Il15*.

En conclusion, nos résultats suggèrent que l'expression de IL15Rα dans les cellules intestinales pourrait être nécessaire au recrutement et / ou au maintien de la population de cellules immunitaires responsable des lésions tissulaires dans l'intestin. La réduction de l'expression des médiateurs pro-inflammatoires dans les entéroïdes déficients en *Il15* indique que l'IL-15 épithéliale régule l'homéostasie des cryptes par la modulation de cytokines et de chimiokines, capables d'activer les leucocytes dans l'épithélium intestinal pour perpétuer l'inflammation.

Mots-clés : IL-15, épithélium intestinal, maladie inflammatoire de l'intestin, inflammation, colite ulcéreuse, IL-15Rα.

ABSTRACT

Role of cytokine IL-15 in the homeostasis of the intestinal epithelium and inflammatory bowel diseases.

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Cell Biology Program

Master's thesis presented to the Faculty of Medicine and Health Sciences in view of obtaining the degree of Master's in science (MSc.) in Cell Biology

Inflammatory bowel diseases (IBD), such as Ulcerative Colitis and Crohn's disease, are characterized by increased intestinal permeability caused by intestinal mucosal barrier dysfunction, a critical factor in the pathogenesis of IBD. The immune system is the major effector of this tissue damage, through the action of multiple cell types as well as soluble mediators, such as TNFα, which are currently therapeutic targets in the treatment of IBD. Interleukin 15 (IL-15) is a pro-inflammatory cytokine involved in maintenance and activation of subgroups of cytotoxic lymphocytes and macrophages in the intestinal epithelium, by acting through the 3-subunit receptor IL-15R. IL-15 receptor complex consists of a unique IL-15R alpha subunit, IL-2/IL-15R beta, and the common gamma-chain/IL-2R gamma subunit. IL-15 expression is increased in IBD and in murine models of intestinal inflammation. However, the exact role of IL-15 signaling in intestinal homeostasis and inflammatory responses, particularly in intestinal epithelial cells (IEC), remains to be determined. The function of IL-15 has been widely described in immune cells, but its functions in other tissues are less well known. The aim of this thesis is to investigate the role of IL-15 in the homeostasis of the intestinal epithelium and in inflammatory bowel diseases.

We tested the influence of IL-15 on intestinal homeostasis in C57BL/6 wild-type (WT), IL-15 deficient (*Il15*$^{-/-}$) and IL-15Rα deficient (*Il15ra*$^{-/-}$) mice. After 7 days, macromorphological analysis of the small and large intestine of *Il15* null mice did not reveal any spontaneous abnormalities when compared to WT littermates. To determine the impact of IL-15 signaling on intestinal proliferation and repair under exogenous stress, we exposed WT and *Il15*$^{-/-}$ mice to dextran sulfate sodium (DSS), a chemical irritant that disrupts the intestinal epithelial barrier and results in induction of colitis. Mice deficient in *Il15* did not display a decreased susceptibility to acute DSS-induced colitis. Additionally, we found that blocking IL-15 signalling on DSS-colitis onset with the TM-β1 antibody, who bind to the β chain of the receptor shared by cytokines IL-2 and IL-15, prevents the overall outcome of the colitis associated inflammation in *Il15ra*$^{-/-}$ mice, but not in WT mice.

Using mouse *in vitro* crypt enteroids from WT and *Il15*-deficient mice, this study demonstrates that exogenous mIL-15 promoted increased mRNA levels of the Lgr5 stem cells marker only in mutant enteroids, but not other epithelial cell linage markers tested, suggesting a role of IL-15 in intestinal cell proliferation and regeneration. Intestinal enteroids stimulated with IFNγ significantly reduced *in vitro* basal proliferation and viability in WT and *Il15*-deficient enteroids. Interestingly, reduced expression of the *Tnfa* and *Cxcl10* genes was observed in *Il15*-deficient small intestinal organoids under IFNγ treatment.

In conclusion, our results suggest that IL15Rα expression in intestinal cells may be needed for the recruitment and/or maintenance of the immune cell population responsible for tissue damage in the intestine. The reduce expression of pro-inflammatory mediators in *Il15*-deficient enteroids indicates that epithelial IL-15 regulates crypt homeostasis through the modulation of cytokines and chemokines, that can activate leukocytes in the intestinal epithelium to perpetuate inflammation.

Keywords: IL-15, intestinal epithelium, inflammatory bowel diseases, inflammation, ulcerative colitis, IL-15Rα.

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LIST OF ABBREVIATIONS

AICD	Activation-induced cell death
AMP	Antimicrobial peptides
APC	Antigen presenting cells
CD	Crohn's disease
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
cDNA	complementary Deoxyribonucleic acid
Chg	Chromogranin
Cxcl10	C-X-C Motif Chemokine Ligand 10
DC	Dendritic cells
DNA	Deoxyribonucleic acid
DSS	Dextran sulfate sodium
EATL	Enteropathy associated T-cell lymphoma
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
ER	Endoplasmic reticulum
FABP2	Fatty acid-binding protein 2
GFP	Green fluorescent protein
GLUT2	Glucose transporter 2
HEPES	Hydroxyethyl piperazineethanesulfonic acid
HRP	Horseradish peroxidase enzyme
IBD	Inflammatory bowel diseases
IEC	Intestinal epithelial cells
IEL	Intraepithelial lymphocytes
IFNβ	Interferon beta
IFNγ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
IL-15	Interleukin 15
$Il15^{-/-}$	IL-15 deficient
IL-15R	Interleukin 15 receptor

Il15ra$^{-/-}$	Interleukin 15 receptor alpha deficient
IL-23R	Interleukin-23 Receptor
ILC	Innate lymphoid cells
JAK	Janus kinase
Lck	Lymphocyte-specific protein tyrosine kinase
Lgr5	Leucine Rich Repeat Containing G Protein-Coupled Receptor 5
LP	Lamina propria
LSP	Long signal peptide
Lyn	Tyrosine-protein kinase
mAb	Monoclonal antiboby
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein-1
MHC	Major histocompatibility complex
mIFNγ	mouse Interferon gamma
mIL-15	mouse Interleukin 15
MLCK	Myosin light chain kinase
mRNA	messenger RNA
MUC	Mucine
Myd88$^{-/-}$	Myeloid differentiation primary response 88 deficient
NF kB	Nuclear factor kappa B
NK	Natural killer
NKT	Natural killer T cells
NLR	Nucleotide-binding oligomerization domain-containing protein -like receptor
NOD	Non-obese diabetic
NOD2	Nucleotide-binding oligomerization domain-containing protein 2
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
PI3-kinase	Phosphatidylinositol 3 kinase
pIgR	Polymeric immunoglobulin receptor
PRR	Pattern-recognition receptors
qPCR	Quantitative real-time polymerase chain reaction
KO	Knockout

$Rag1^{-/-}$	Recombination-activating gene 1-deficient
RCD	Refractory Celiac Disease
RegIII	Regenerating islet-derived protein III
RLR	RIG-I-like receptor
RNA	Ribonucleic acid
ROR	Tyrosine kinase-like orphan receptor
SCID	Severe combined immunodeficient mice
sIL-15Rα	soluble Interleukin 15 receptor
Sis	Sucrase-isomaltase
SMAD	Mothers against decapentaplegic homolog
SSP	Short signal peptide
STAT	Signal transducer and activator of transcription
Syk	Spleen tyrosine kinase
T-bet	T-box transcription factor TBX21
TCR	T-cell receptor
TGFα	Transforming growth factor alpha
T_H	T helper
TLR	Toll-like receptor
TNFR	Tumor necrosis factor Receptor
TNFα	Tumor necrosis factor alpha
TNFβ	Tumor necrosis factor beta
Treg	Regulatory T cells
TSLP	Thymic stromal lymphopoietin
UC	Ulcerative colitis
WNT3	Wingless-type MMTV integration site family, member 3
WT	Wild-type

1. INTRODUCTION

1.1. The digestive system

The digestive system consists of the gastrointestinal tract and accessory organs of digestion necessary for nutrient assimilation and degradation, including mouth, pharynx, esophagus, stomach, small intestine and colon that ends with an orifice, the anus. Indeed, digestion degrades food into nutrients to be absorbed through the intestinal epithelial lining into the bloodstream, before disposal of residual products (Marieb and Hoehn, 2015). Small intestine and colon are separated by the appendix in humans and the *caecum* in mice. The intestine, consisting of the small intestine and colon, constitutes most of the digestive tract and is the site of food digestion and absorption.

1.1.1. The small intestine

Following the stomach, the small intestine, the main place where digestion and selective absorption of nutrients occurs, produces hormones, enzymes and mucus. There are three main regions: the duodenum, the jejunum and the ileum. At first, the chyme (food) of the stomach mixes with pancreatic juices and bile in the jejunum and becomes less acidic. Then, nutrients produced are mainly captured in the jejunum. Finally, bile salts and vitamins are absorbed in the ileum (Smith and Morton, 2010).

1.1.2. The colon

The colon is the second segment of the intestine after the ileum. Its main functions are to store fecal material, regulate its release into the external environment and absorb water and electrolytes from the chyme, resulting in a more solid fecal material (Smith and Morton, 2010). A mixture of bile and a thick mucus lubricates the passage of the fecal material, providing a growth environment for different families of microorganisms, some of which synthetize an important part of the vitamin requirement of the body (Gracia-Sancho and Salvadó, 2017). The colon is divided into four main regions, including the ascending, transverse, descending and sigmoid colon. However, for practical reasons, it is usually separated into proximal and distal colon in mice. The colon ends with the rectum, which is in contact with the anus.

1.1.3. General structure of the intestine

The intestine is composed of different segments, each with a specific function. The general structure of the intestinal wall is preserved between these segments. The intestinal wall is divided into four different sections: the mucosa, the submucosa, the external muscle and the serosa (Fig. 1A, 1C) (Young, 2006).

The mucosa is the layer directly in contact with the intestinal lumen and is composed of the epithelium, lamina propria (LP), muscularis mucosae, and submucosa, a smooth muscle layer. The epithelium is a single layer of epithelial cells lining the entire intestinal tissue. The lamina propria contains the blood and lymphatic vessels necessary for the absorption of nutrients and oxygenation. Finally, the muscularis mucosae contains the muscle cells allowing peristaltic movement of the gut. The submucosa, formed by the connective tissue, contains blood vessels and the nervous plexus. The external muscle layer is composed of a succession of internal circular and longitudinal smooth muscles, which also allow peristalsis that propels food chyme along the digestive tract. Finally, the entire wall is surrounded by serosa. The serosa is composed of a layer of connective tissue which protects the intestinal wall by preventing possible friction or displacements (Smith and Morton, 2010).

1.2. The intestinal epithelial barrier

The epithelial surface of the intestine is the largest of all mucosal surfaces in the human body, comprising about 400 m^2 of a single layer of epithelial cells. These epithelial cells constitute the crypt and villi compartments in the small intestine and the crypts and surface epithelium in the large intestine. Pluripotent intestinal epithelial stem cells, present at the base of the crypt, are responsible for the continued renewal of the epithelium. This local stem cell niche contributes to the generation of absorptive cells, goblet cells, enteroendocrine cells and Paneth cells through proliferation and differentiation of epithelial cell progenitors (Fig. 1B). However, Paneth cells are absent from the colon (Fig. 1D) (Crosnier *et al.*, 2006; van der Flier and Clevers, 2009). In addition, the diversity of functions carried out by the intestinal epithelium is reflected by the presence of additional specialized intestinal epithelial cell (IEC) lineages, such as M

cells and *tuft* cells, also present in the colon, while *cup* cells are predominantly restricted to the ileum (Fujimura and Iida, 2001).

1.2.1. Absorptive cells

Enterocytes and colonocytes are the most abundant cell type of the intestine, representing 80% of the all IEC (van der Flier and Clevers, 2009). They have a cylindrical and polarized shape, in addition to having a brush border composed of microvilli on their apical surface (Noah *et al*., 2011). This structure increases the area of the contact surface with the nutrients, to favor their absorption and then to release them on the basolateral side for transportation into the lymphatic and blood vessels.

They also express many digestive enzymes including sucrase-isomaltase and alkaline phosphatase, which cleave disaccharides and control lipid absorption, respectively (Ferraris *et al*., 1992). They also produce proteins, such as Fatty acid-binding protein 2 (FABP2) that binds and promotes the absorption of fatty acids (Gajda and Storch, 2015), and Glucose transporter 2 (GLUT2), which promotes glucose and fructose paracellular transport (Kellett *et al*., 2008). Colonocytes, in addition to the absorption of water and salts, also secrete β-defensins and type-C lectins like RegIIIβ and RegIIIγ, which act as antimicrobial peptides (AMP) (Cobo and Chadee, 2013; van Ampting *et al*., 2012).

1.2.2. Paneth cells

Paneth cells are found exclusively in the crypts (Fig. 1E), in the bottom compartment of the crypt-villus axis and are normally not present in the colon. Paneth cells are secretory cells characterized by an apical cytoplasm filled with very dense granules containing AMP, such as lysozyme and α-defensins, that protect the host from enteric pathogens (Wilson *et al.*, 1999) and modulate the composition of the intestinal microflora (Biswas *et al*., 1999). In addition to AMP secretion, Paneth cells are also important for maintaining the stem cell niche at the base of the crypts. Paneth cell-mediated expression and secretion of growth factors, such as transforming growth factor-α (TGFα), Epidermal growth factor (EGF) and Wingless-type MMTV integration site family, member 3 (WNT3) in this niche, and interaction with stem cells to determine cell fate, ensures IEC integrity (Sato *et al.*, 2011).

1.2.3. Enteroendocrine cells

Enteroendocrine cells are one of the less represented cell types in the intestine, accounting for about 1% of IEC in the small intestine and colon. Enteroendocrine cells, responsible for secretion of different hormones that regulate the digestive function (Noah *et al.*, 2011), are found along the mucosa, often isolated from each other by non-endocrine IEC (Fig. 1E) (Buffa *et al*., 1978). There are more than 16 enteroendocrine cell subtypes identified in the murine gut (Evans et Potten, 1988) that secrete more than 30 different digestive hormones. These hormones include cholecystokinin, secretin, motilin and chromogranin (Chg) A and B (Gunawardene *et al.*, 2011).

1.2.4. Goblet cells

Goblet cells, the most abundant cells of the secretory lineage of the intestinal epithelium (Fig. 1E) (Noah *et al.*, 2011), produce and secrete the mucus that acts as a line of defense against xenobiotics, microbes or viruses present in the intestinal lumen (Dorofeyev *et al*., 2013). Mucus is a gel-like structure composed of different mucins, which are large glycoproteins with multiple oligosaccharide chains. Mucins are secreted by goblet cells, either constitutively or in a regulated manner in response to exogenous factors, such as pro-inflammatory cytokines or bacteria (Lindén *et al*., 2008). Of the different mucins, MUC2 is secreted while MUC1 and MUC3 are membrane-associated. The intestinal mucus is mainly composed of MUC2, which is more abundant in the colon. The mucus is organized in the small intestine as a single layer or as a double layer in the colon. The colon inner layer, immediately adjacent to the epithelium, is stratified and forms a filter that physically separates IEC from luminal bacteria (Johansson *et al.*, 2008). The spontaneous development of colitis and the predisposition to inflammation-induced colorectal cancers observed in MUC2-deficient mice highlights the importance of mucin production by goblet cells (Van der Sluis *et al*., 2006; Velcich *et al*., 2002).

1.2.5. Stem cells and proliferative progenitor cells

Constant divisions of stem cells at the base of the crypts renew the intestinal epithelium every three to five days (Barker, 2013). Following an asymmetric division, a stem cell gives rise to another stem cell and a progenitor cell (Fig. 1E). Progenitor cells migrate towards the top of the crypt, dividing four or five times during the process (van der Flier and Clevers, 2009). Progenitor cells receive signals of differentiation, leading to growth arrest and cell differentiation to generate each of the differentiated IEC types listed above, each with its specialized function (Bloemendaal *et al*., 2016).

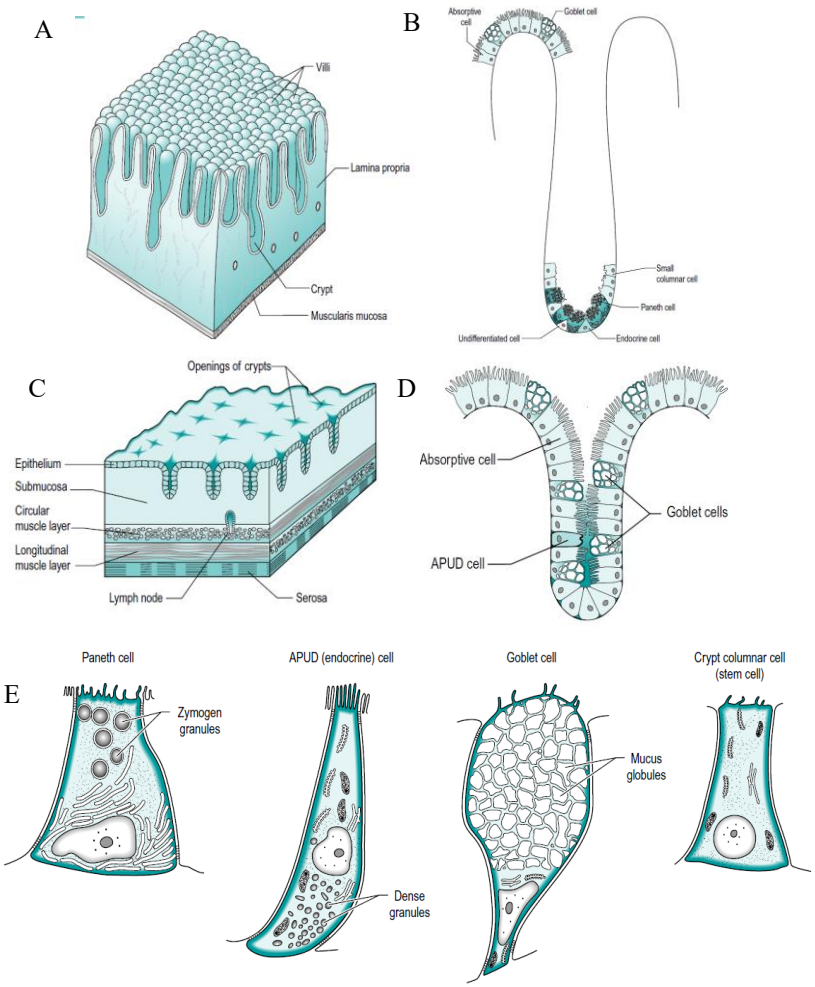

Figure 1. The intestinal epithelial barrier (A) Structure of the submucosa of the small intestine, showing the relationship of the glands with the crypts to the villi. **(B)** Localization of the different epithelial cell types along the crypt-villus axis. **(C)** Structure of the wall of the colon, with the different layers indicated. **(D)** Cell types in the colon epithelium. **(E)** From left to right: Paneth cell, Endocrine cell, Goblet cell, Stem Cell. (Adapted from Smith and Morton, 2010, License#4647130728131).

1.3. Maintenance of intestinal homeostasis

The intestinal epithelium is a rapidly renewing organ in constant contact with the microbiota and the immune system. It is important to maintain intestinal integrity and homeostasis on an ongoing basis in order to prevent the appearance of pathologies, such as inflammatory bowel diseases (IBD). To this end, the epithelium provides different levels of defense against commensal bacteria contained in the intestinal lumen.

1.3.1. Biochemical barrier

Luminal bacteria derived from more than 500 different species colonize the intestinal mucosa (Macpherson and Harris, 2004). One of the roles of the mucosal barrier is to locally defend against environmental threats (e.g. invading pathogens). At the same time, the mucosal barrier is impermeable to most hydrophilic solutes in the absence of specific transporters. The mucus and epithelial layer form a strong line of defense against toxic compounds, including bacteria and their products, viruses and dietary components from the intestinal lumen (Pearson and Brownlee, 2010).

The thick layer of mucus is composed of a dense glycocalyx with mucins associated with the membrane and impermeable to most bacteria. This biochemical barrier constitutes the first line of defense at the intestinal and mucosal interface. Intestinal mucus also contains high concentrations of antimicrobial molecules like the antimicrobial peptides (AMPs), produced by both absorptive cells and Paneth cells, and secretory Immunoglobulin A (IgA), which neutralize toxins and germs contained in the intestinal lumen and which are transported by IEC (Yamamoto and Matsumoto, 2016).

1.3.2. Physical barrier

The epithelium acts as a physical barrier that establishes segregation between the microbiota and the intestinal mucosa. A balance between cell proliferation, differentiation, migration along the crypt-villus axis and elimination by anoïkosis is, therefore, essential to maintain intestinal homeostasis (Bjerknes and Cheng, 2005). IEC, thus, regulate colonization and penetration of bacteria in the epithelium (Maloy and Powrie, 2011) and seal the intercellular space through tight junctions, adherent junctions

and desmosomes. These junctions consist of transmembrane proteins that interact with neighboring cells as well as intracellular adapter proteins linked to the cytoskeleton (Groschwitz and Hogan, 2009).

How does the epithelial cell recognize bacteria? On their cell surface and in the lumen, IEC express different classes of receptors recognizing pathogen specific molecules, collectively referred to as pattern-recognition receptors (PRR). PRR, such as Toll-like receptor (TLR) (Abreu, 2010), NOD-like receptor (NLR) (Chen and Núñez, 2011; Elinav *et al*., 2013) and RIG-I-like receptor (RLR) families (Broquet *et al*., 2011; Loo *et al*., 2011) represent distinct pathways for the recognition of microbial ligands or endogenous signals in IEC. These microbial product receptors play an important role as dynamic sensors of the microbial environment and as active participants in the regulation of mucosal immune cell responses.

In addition to IEC-mediated biochemical and physical barriers, the mucosal lamina propria also comprises many types of immune cells with multiple functions. For example, approximately 70% of all lymphocytes of the human body, and the largest pool of tissue macrophages, are located in the intestinal wall. Therefore, the intestinal mucosal surface plays the intricate role of maintaining a delicate balance between providing protection from infectious agents while retaining immune tolerance to commensals (Macpherson and Uhr, 2004).

1.3.3. Immune cells

A wide variety of immune cells contribute to maintain the integrity of the intestinal mucosa. To ensure optimal nutrient transport while avoiding translocation of bacteria, the intestinal mucosa is equipped with a well-developed immune system. The majority of immune cells and organized lymphoid structures, such as Peyer's patches and isolated lymphoid follicles, are located immediately under the epithelium in the lamina propria, and include macrophages, dendritic cells (DC) and innate lymphoid cells (ILC).

Macrophages and DC are derived from the precursors of the mononuclear phagocytic system (Varol *et al.*, 2007). Differentiated macrophages are resident in the intestinal tissue and participate in antigen presentation, in addition to phagocytosing dead cells and debris, thereby participating in repair and remodeling of tissue lesions following an inflammatory tissue damage (Wynn and Vannella, 2016). Intestinal DC are generally derived from circulating monocytes (Varol *et al.*, 2007), and may migrate to mucosal sites along epithelial cell-derived chemokine gradients (Cook *et al.*, 2000).

ILC are recently identified innate lymphoid cell populations playing a key role in intestinal immune homeostasis. ILC, located at barrier surfaces, such as skin (Kim *et al.*, 2013), lung (Monticelli *et al.*, 2011), and intestine (Tait Wojno and Artis, 2012), are regulated in part, by epithelial cell-derived immunoregulatory signals, and are characterized by their developmental lineage and differential cytokine expression profiles. Classified group 1, group 2 and group 3 ILC share functional similarities with T helper (T_H) cells of the adaptive immune system, namely $CD4^+$ T_H1, T_H2 and T_H17 cell populations, respectively (Spits and Cupedo, 2012)

IgA-secreting B cells are also implicated in adaptive immune responses at the epithelial surface and contribute to the maintenance of the epithelial barrier. Plasma cells, present in the lamina propria, are the major IgA producers. At the IEC basolateral membrane, dimeric IgA complexes bound by the polymeric immunoglobulin receptor (pIgR) are actively transcytosed into the intestinal lumen (Johansen and Kaetzel, 2011). Thus, IEC-directed transport of secretory immunoglobulins across the epithelial barrier regulates commensal bacterial populations in order to maintain epithelial and immune cell homeostasis (Finn-Eirik Johansen *et al.*, 1999; Shulzhenko *et al.*, 2011; Suzuki *et al.*, 2004).

The intestinal immune system also includes mature T cells subject to the direct influence of IEC for their survival and functional maintenance in the lamina propria. Intraepithelial lymphocytes (IEL) are specialized cells found in the mucosal epithelial lining or in structures, such as mesenteric lymph nodes (Tomasello and Bedoui, 2013). It has been estimated that an average of 10 to 20 IEL per 100 enterocytes are present in the human

small intestine (Crowe and Marsh, 1994). IEL present an activated phenotype and include conventional T cells as well as subsets of cells expressing a restricted repertoire of T cell receptor specificities and specialized properties, including γδ T cells and Natural killer T (NKT) cells (Cheroutre *et al*., 2011; Ismail *et al.*, 2011). Bidirectional interactions between IEL and IEC, involved in local immunosurveillance, maintain immune homeostasis at the intestinal barrier (Yu *et al.*, 2006)

It is important that the intestinal epithelium does not mount a pro-inflammatory immune response in the presence of commensal bacteria beneficial to the host. However, the mechanisms by which the immune system of the intestinal mucosa maintains tolerance in the presence of commensal bacteria are not yet completely elucidated. One possible explanation of this tolerance involves restricted IEC localization of TLR responsible for recognition of microbial motifs. For example, the expression of TLR5, which recognizes flagellin, is restricted to IEC basolateral surfaces and is therefore only activated in the presence of invading bacteria in the lamina propria (Gewirtz *et al*., 2001).

1.4. Inflammatory bowel diseases

Inflammatory bowel diseases (IBD), a group of pathologies affecting the gastrointestinal tract, include Crohn's disease (CD) and ulcerative colitis (UC). IBD are characterized by severe episodic intestinal inflammation, with diarrhea, abdominal pain and rectal bleeding in patients. Extra-intestinal complications such as arthritis, osteoporosis and anemia are also common (Stein *et al*., 2010). In 2018, the Crohn's and Colitis Foundation estimated that 270,000 Canadians are living with IBD, making Canada one of the countries most affected by these pathologies. The prevalence of IBD in Canadian children has risen by more than 50% in the last 10 years.

1.4.1. Crohn's disease and ulcerative colitis

Despite some similarities, CD and UC differ mainly in the anatomical location and the severity of the observed inflammation. CD affects any part of the digestive tract from mouth to anus, but more frequently attacks the terminal ileum. Inflammation is transmural and discontinuous, crossing all the layers of the intestinal wall, and is localized to certain regions alternating with healthy regions. Histologically, it is often associated

with the presence of granulomas, which correspond to an aggregation of macrophages (Xavier and Podolsky, 2007). UC affects mainly the colon and often follows a gradient in severity from proximal to distal regions, and the terminal colon is more severely affected. The observed inflammation is rarely transmural but is rather concentrated in the mucosa and the submucosa. Histologically, ulcerations and cell loss are frequently observed. IBD is an idiopathic condition, which means that we do not still know the exact causes. However, it is known that a strong genetic component is involved (Xavier and Podolsky, 2007). There is also evidence that environmental factors such as diet components and microbiota play an important role in the pathogenesis of IBD (Nielsen and Ji, 2015; Rolhion and Chassaing, 2016).

1.4.1.1. Intestinal microbiota

Many studies suggest that gut microbiota abnormalities, such as the loss of diversity (reduction in *Bacteroides*, *Eubacterium*, and *Lactobacillus spp* in combination with an increase in *Bacteroidetes* and *Proteobacteria*) (Chassaing and Darfeuille–Michaud, 2011; Frank *et al.,* 2007; Nishikawa *et al.,* 2009; Swidsinski *et al.*, 2002) appear to be primary events that contribute to the inflammation process (Blaut and Clavel, 2007). This alteration in the composition and function enhance a decreased production of short chain fatty acids by gut microbiota, mainly butyrate, who appears to be a key factor in the balance between inflammation and immunosuppression in the intestinal tract, such as the suppression of the activation of NF-κB and activation of Treg cells, responsible for peripheral tolerance (Silva *et al.*, 2018). Among others, smoking, stress and diet are other important environmental factors (Ananthakrishnan, 2015). IBD are considered as multifactorial diseases in which genetically predisposed individuals show an exacerbated immune response to environmental or microbial stresses, and which are linked to several risk factors affecting the integrity of the intestinal barrier and immune response, among others (Ananthakrishnan, 2015; Anderson *et al.,* 2011; Kaser *et al.,* 2010).

1.4.1.2. Intestinal barrier

The physical integrity of the epithelial barrier may be compromised in IBD. Indeed, an increase in paracellular permeability has been documented in areas of chronic acute

inflammation in patients with IBD (Schmitz *et al.*, 1999). Animal studies support the idea that inflammation develops in areas where permeability is affected and reciprocally, the permeability increase could be a triggering event for this inflammation (Madsen *et al.*, 1999).

The single layer of epithelial cells displays a strict balance between proliferation and apoptosis to maintain the intestinal barrier (Edelblum *et al.* 2006). If cell death is not finely regulated, this can result in a breach in the barrier, resulting in an elevation in permeability, microbial invasion and inflammation. In this respect, some studies have shown that IEC proliferation and renewal are accelerated in IBD, with programmed cell death being especially higher in CD than UC patients (Di Sabatino *et al*., 2011). Interferon gamma (IFNγ) and Tumor necrosis factor -α (TNFα) are two cytokines known to regulate IEC proliferation and apoptosis (Kaiser and Polk, 1997; Ruemmele *et al.,* 1998).

Although the permeability defects seen in patients with IBD could possibly be caused by the marked apoptosis that occurs during the process of inflammation, many studies have clearly shown that IEC apoptosis alone is not responsible for the permeability loss (Laukoetter *et al*., 2008). It has been shown that the expression and localization of proteins comprising the cell junctions are severely affected in IBD patients following activation of the mucosal immune system (Landy *et al.*, 2016).

Goblet cells play an important role in protecting the intestinal epithelium. Defects in these cells are often observed in patients with IBD. In CD, early hyperproduction of mucus and abnormal glycosylation are frequent events. More precisely, patients with CD have moderate or increased expression of mucins in addition to having an increase in their sulfation, resulting in a thicker mucus layer, while the mucus layer is much reduced in UC (Dorofeyev *et al.*, 2013).

1.4.1.3. Genetic predisposition

The first gene associated with CD is Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) (Hugot *et al.*, 2001). Signaling through the NOD2 protein activates nuclear factor Nuclear factor kappa B (NF-kB), which takes part in many

signaling cascades that stimulate the transcription of pro-inflammatory genes and protective molecules (Sartor, 2006). NOD2 is expressed constitutively in Paneth cells and is necessary to produce α-defensins protect the epithelium against infections (Kobayashi *et al.*, 2005; Lala *et al.*, 2003). Also, a critical association between IBD and the Interleukin-23 Receptor (IL23R) gene has also been described (Duerr *et al.*, 2006). The IL23R gene encodes a subunit of the IL-23 receptor, which is activated by the IL-23 proinflammatory cytokine involved in the generation of T_H 17 cells (McGeachy and Cua, 2007). Several genes associated with the IL-23/T_H17 signaling cascade have also been identified as susceptibility loci for IBD, namely IL12B, Janus kinase (JAK) 2 and Signal transducer and activator of transcription (STAT) 3 (Barrett *et al.*, 2008; Jostins *et al.*, 2012). Polymorphisms in TLRs are other mechanisms that could support a dysfunctional immune system promotes abnormal inflammatory response to enteric bacteria (Shen *et al.*, 2010; Zhang *et al.*, 1999).

1.4.1.4. Immune response

Imbalance between effectors and regulators of immune cell populations results from an altered immune response to enteric elements (Ordás *et al*., 2012; Sun *et al*., 2015) and altered production of inflammatory mediators (Heller *et al*., 2005). In IBD patients as well as in murine models of experimental intestinal inflammation, pro- and anti-inflammatory cytokines have been shown to be produced by IEC and a variety of mucosal immune cells. Cytokines form a broad family of glycoproteins that influences various physiological process, that range from hematopoiesis to immune responses (Krebs and Hilton, 2001). In the intestine, immune cytokine-producing cells are DC, neutrophils, macrophages, innate lymphoid cells and effector and regulatory helper T cells. T cells are involved in the pathogenesis of IBD as shown by the high number of T cells detected in the inflamed intestinal wall and by T-cell dependent inflammation observed in several animal models (Neurath, 2014). Several subgroups of T cells can be discerned according to the secreted cytokines and their influence on the development of inflammation, notably T_H1, T_H2, T_H17 cells with effector functions, and regulatory T cells (Treg) with regulatory functions.

Several immunoregulatory signals essential for tolerizing immune cells, and for regulating innate and adaptive immune cell responses against pathogens and commensal bacteria as well as steady-state inflammation, are produced by IEC. IEC-derived thymic stromal lymphopoietin (TSLP), TGFβ and retinoic acid, produced in response to commensal bacteria-derived signals, induce the development of DC and macrophages with tolerogenic properties characterized by production of Interleukin 10 (IL-10) and retinoic acid (Rimoldi *et al*., 2005; Zeuthen *et al*., 2007). Under homeostatic conditions, tolerance in the intestine is controlled by a wide array of independent immunosuppressive mechanisms with partially overlapping functions. Several types of regulatory lymphocytes exert their function in the intestine through a relatively small number of known mechanisms common to several cell lineages. The best characterized to date are the cytokine IL-10 and TGFβ (Izcue *et al*., 2009; Kole and Maloy, 2014).

IL-10, a homodimeric anti-inflammatory cytokine produced by most hematopoietic and some non-hematopoietic cell types, acts on lymphocytes and myeloid cells to suppress a wide range of immune responses. IL-10 can be induced on some non-hematopoietic cells and is constitutively expressed in the colonic epithelium (Moore *et al*., 2001). Even though IL-10 can moderate different inflammatory pathways, IL-10 knockout mice do not suffer from a severe lethal autoimmune syndrome, but instead spontaneously develop colitis in the presence of triggering microorganisms, which highlight a crucial role of cytokines in intestinal inflammation (Kühn *et al*., 1993). Additionally, studies in experimental mouse models of IBD have shown that both the neutralization of pro-inflammatory cytokines and the administration of recombinant anti-inflammatory cytokines can prevent chronic intestinal inflammation (Neurath *et al*., 1995; Powrie *et al*., 1994; Strober *et al*., 2002). Mice inactivated for the genes encoding IL-10 or TGFβ, lack functionally active Treg cells, fail to suppress pro-inflammatory cytokine production by Antigen presenting cells (APC) and effector T cells, and spontaneously develop chronic intestinal inflammation, highlighting the functional importance of these anti-inflammatory cytokines (Asseman *et al*., 1999; Huber *et al*., 2011; Powrie *et al*., 1996).

The pro-inflammatory functions of mucosal macrophages and effector T cells are suppressed by Treg producing TGFβ, which lead to the intracellular activation of SMAD3 and SMAD4 proteins. Overexpression of SMAD7, which inhibits TGFβ signaling, by effector T cells from IBD patients may confer resistance to TGFβ-mediated suppression (Fantini *et al*., 2009; Monteleone *et al*., 2001; Rani *et al.,* 2011). Based on

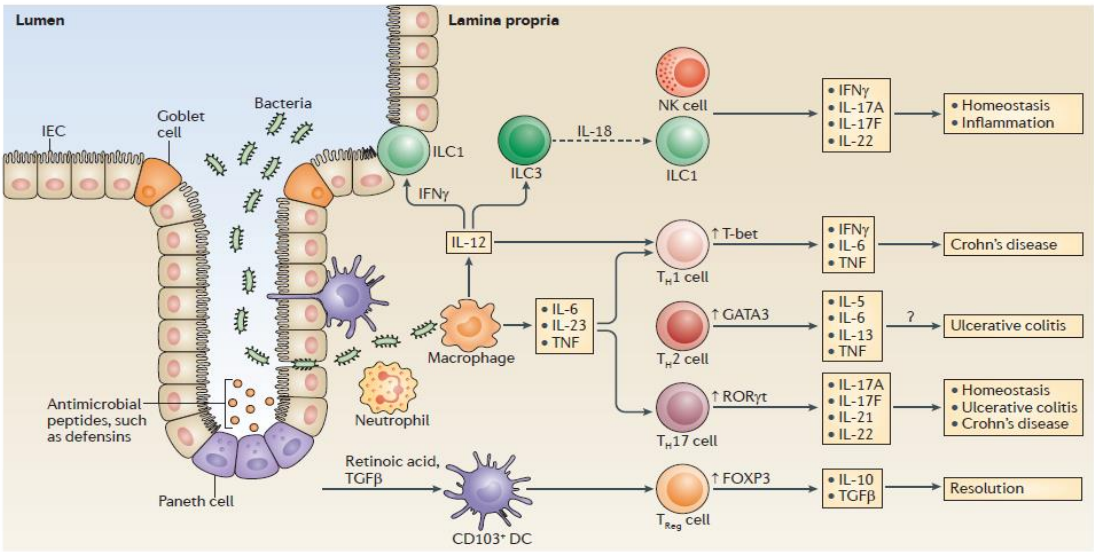

Figure 2. Influence of cytokines in the pathogenesis of IBD Pro-inflammatory and anti-inflammatory cytokines are produced by various cells of the mucosal immune system in response to environmental triggers, in experimental mouse models of colitis and in patients with inflammatory bowel diseases (IBD). Dendritic cells (DC), neutrophils, macrophages, natural killer (NK) cells, intestinal epithelial cells (IEC), innate lymphoid cells (ILC), as well as auxiliary T cells T_H1, T_H2, T_H17 and Treg produce cytokines in the inflamed mucosa. While the Th1 subgroup promotes a phenotype more associated with CD and that the Th2 subtype is more associated with UC, the subtype Th17 is associated with both CD and UC (From Neurath, 2014, License#4611410642217).

this assumption, SMAD7 antisense oligonucleotides, tested as a new therapeutic agent in CD patients, have shown remarkable beneficial effects in a Phase I clinical study (Monteleone *et al.*, 2012). However, treating patients with anti-inflammatory cytokines IL-10, IFNβ and IL-11 was not effective in controlling the pathology associated with IBD (Herrlinger *et al*., 2006; Musch *et al*., 2005; Tilg *et al*., 2002).

As shown in Figure 2, the T_H1 subgroup also produces cytokines such as IFNγ and IL-6. In CD patients, the production of IFNγ by cells of the lamina propria and lymph node T

cells is increased compared with the production in healthy controls patients (Breese *et al*., 1993; Fais *et al*., 1991; Sakuraba *et al*., 2009). The key regulators T-bet and Signal transducer and activator of transcription 4 (STAT4) are responsible for T_H1 cell differentiation (Lazarevic *et al*., 2013; Szabo *et al*., 2002; Wirtz *et al*., 1999). STAT4 deficient mice are protected from experimentally induced colitis, whereas STAT4 overexpression exacerbates colitis, suggesting that T_H1 cells are promoters of IBD pathology (Neurath *et al.*, 2002; Wirtz *et al.*, 1999). IFNγ and TNF have been also shown to alter tight junction activity and to induce IEC apoptosis (Nava *et al.*, 2010; Su *et al.*, 2013). However, while specific IFNγ antibodies were therapeutically effective in a T cell transfer model of colitis (Powrie *et al.*, 1994), treatment of CD patients with the IFNγ-specific antibody fontolizumab had no such effect (Reinisch *et al.*, 2006), suggesting that targeting a single T_H1-type cytokine might not be effective clinically.

Another T_H1 cytokine with pro-inflammatory functions is IL-6, which can activate multiple target cells, including APC and T cells, preventing apoptosis of mucosal T cells and activating pro-inflammatory cytokine production by these cells (Atreya *et al.*, 2000).

Pro-inflammatory cytokine TNFα exerts various functions by binding to receptors TNFR1 and TNFR2 followed by the intracellular activation of NF-κB. TNFα exists as both membrane-bound and soluble TNFα, and its production by lamina propria mononuclear cells is markedly increased in patients with IBD. In colitis, TNFα signaling enhances pleiotropic pro-inflammatory effects, including increased angiogenesis, Paneth cell death induction via necroptosis, matrix metalloproteinase production by myofibroblasts, activation of effector T cells and macrophages, and direct damage of IEC via myosin light chain kinase (MLCK) activation (Atreya *et al*., 2011; Di Sabatino *et al*., 2007; Günther *et al*., 2011; Meijer *et al*., 2007; Su *et al*., 2013). Based on recent clinical studies showing that membrane-bound TNFα, rather than soluble TNFα, plays a major role in driving intestinal inflammation, neutralization of membrane-bound TNFα has been effective in suppressing experimental colitis in mice by inducing T cell apoptosis. Additionally, antibodies that bind both soluble TNFα and membrane-bound TNFα (such as infliximab and adalimumab) were highly effective in IBD treatment by inducing T cell apoptosis, whereas agents that preferentially neutralize soluble TNFα (for example,

Etanercept) had no therapeutic effect (Atreya *et al*., 2011; Brande *et al*., 2007; Van den Brande *et al*., 2003).

ILC also regulated, in part, by epithelial cell-derived immunoregulatory signals, are an important source of IFNγ and IL-23-inducible pro-inflammatory cytokines, such as IL-17A and IL-17F, which facilitate experimental innate immune-mediated colitis (Buonocore *et al.*, 2010). T_H17-type cytokines, such as IL-17 and IL-21, have some pro-inflammatory roles including neutrophil recruitment, matrix metalloproteinase secretion by intestinal fibroblasts and upregulation of TNFα, IL-1β, IL-6 and IL-8 (Monteleone *et al*., 2006; Monteleone *et al*., 2005; Siakavellas and Bamias, 2012). T_H17 cells may also produce anti-inflammatory cytokines, such as IL-22, controlling IEC proliferation, the production of mucins and antimicrobial proteins, such as defensins, REGIIIβ and REGIIIγ proteins, via STAT3 activation, and wound healing (Pickert *et al.*, 2009). Studies in mouse models of experimental colitis have shown that absence or neutralization of IL-17A or IL-17F alone had no effect, or even aggravated disease activity, in a T cell transfer model of colitis (O'Connor Jr *et al.*, 2009).

Recent studies have advanced the understanding of the developmental origin of committed $CD4^+$ T cells and their functions at the intestinal barrier in IBD (Nemoto *et al.*, 2007). T_H1 cells produce cytokines such as IFNγ and IL-6. The T_H1 profile is therefore, more associated with a CD disease-like phenotype (Neurath, 2014). T_H2 cells secrete cytokines such as IL-5 and IL-13 and express the *GATA3* transcription factor, which are all increased in UC patients (Neurath, 2014). T_H17 cells strongly express *IL17,* the surface marker *IL23R* and the transcription factor *RORγt* both in CD and UC patients. T_H17 cells also produce IL-6 and IL-23 whose expression is increased in response to the microbiota (Fig. 2) (Ivanov *et al.*, 2009).

In the lamina propria, conventional effector T cells also exert a tolerogenic or inflammatory effect on the local environment by directly influencing IEC. Bidirectional interactions between IEL and IEC have a role in preserving immune homeostasis at the intestinal barrier (Cheroutre *et al.*, 2011; Edelblum *et al.*, 2012; Yu *et al.*, 2006). This seems to be particularly important in $CD8^+$ T cell-dependent memory responses, present

in the intestinal compartment of humans and mice (Gebhardt *et al*., 2013; Sathaliyawala *et al*., 2013).

1.4.2. Experimental models of IBD

Animal models of intestinal inflammation have been used to understand mucosal immunology, immune homeostasis, maintenance of intestinal homeostasis and intestinal inflammation in relation to IBD. Each model offers valuable insights into one or another major aspect of the disease, but no single model captures the complexity of human IBD.

Hermiston and Gordon, in an influential study published in the 1990s, demonstrated the importance of an intact epithelium in the prevention of mucosal inflammation (Hermiston and Gordon, 1995). In this study, a transgenic N-cadherin mutant chimeric mouse with small intestinal patches of villi with poorly adherent and incompletely differentiated enterocytes was used. In these mice, inflammation occurred in the lamina propria in areas under the defective epithelium, suggesting that the inflammatory response was induced by the entry of commensal microorganisms in the lamina propria. Dextran sulfate sodium (DSS), a sulfated polysaccharide, produces a similar effect in mice by causing epithelial cell injury, resulting in immune response and mucosal barrier function alterations through the colonic epithelium. Administration of DSS in drinking water for a short period of time induces reproducible acute inflammation limited to the colon and characterized by erosions/ulcers, loss of crypts and infiltration of granulocytes (Cooper *et al*., 1993; Okayasu *et al*., 1990). Importantly, in severe combined immunodeficient mice (SCID) (Dieleman *et al.*, 1994) and *Rag1*$^{-/-}$ mice (Krieglstein *et al.*, 2002), where adaptive immunity mediated by T cells is absent, the development of inflammation suggests that effector cytokines produced by innate immune cells are sufficient to cause inflammation. On this basis, the DSS model has become a useful colitis model to study the immune mechanisms mediated by innate immune cells, such as IEC, macrophages and neutrophils, during the development of intestinal inflammation (Chami, 2014; Dieleman *et al*., 1994; Fukata *et al*., 2005). In summary, proinflammatory cytokines, as well as cytokines produced by macrophages and neutrophils that regulate epithelial barrier function, are important contributors to tissue damage.

The DSS-induced colitis model has also been used to study the role of innate immune mechanisms initiated by TLR and dectin receptors and inflammasomes (Abreu *et al*., 2005; Rakoff-Nahoum *et al*., 2004). *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, and *Myd88*$^{-/-}$ mice exhibit exacerbation of DSS colitis due to defects in these innate signaling components in IEC and macrophages (Cario *et al*, 2007; Fukata *et al*., 2005; Rakoff-Nahoum *et al*., 2004). More recently, the DSS colitis model has provided a platform for the study of the effects of the gut microbiome on colitis development. This is exemplified by the fact that NOD2 abnormalities in mice give rise to altered susceptibility to DSS colitis, associated with, or perhaps caused by, changes in the gut microbiome (Maeda *et al.*, 2005; Natividad *et al.*, 2012).

Even though activation of macrophages and neutrophils in DSS-induced colitis plays a major role, it should be noted that T-cell responses can exacerbate the inflammatory response when both innate and adaptive immunity are intact. A polarized T_H1 response has been observed in acute colitis whereas a mixed T_H1/T_H2 response was found in chronic forms of DSS colitis, achieved by repeated cycles of DSS administration (Dieleman *et al.*, 1998).

The *in vitro* study of the pathogenesis of IBD needs a cell model demonstrating as closely as possible, the characteristics of the intestinal epithelium *in vivo* . In 1996, continuously growing “normal” human IEC with the ability to express specific cytokeratins as well as IEC markers over several passages were established (Perreault and Beaulieu, 1996). On the other hand, human colon cancer cell lines, such as Caco-2 cells which spontaneously differentiate (Pinto *et al*., 1983; Vachon and Beaulieu, 1992), have been used as experimental models to study various aspects of intestinal functions including cell regulation, cytokine responses and barrier integrity (Beaulieu and Quaroni, 1991; Hauri *et al*., 1985). However, it is obvious that their cancerous nature as well as their colonic origin constitute a limitation of these models (Ménard and Beaulieu, 1994; Rousset, 1986).

The recent use of three-dimensional (3D) intestinal organoid cell cultures has emerged as a new strategy for modelling the intestinal tract with normal proliferating and

differentiating IEC. Organoids have been established to better reproduce tissue characteristics in both normal and diseased physiological conditions (Clevers, 2016; Dedhia *et al*., 2016). An organoid is defined as a structure grown from stem cells and involving organ-specific self-organized cell types that and spatially restricted lineage commitment (Lancaster and Knoblich, 2014; Nakano *et al*., 2012). Important progress has been completed to define optimal conditions allowing growth, expansion and differentiation of intestinal epithelial stem cells (Sato *et al*., 2011) as well as other types of stem cells (Dye *et al*., 2015; Fatehullah *et al*., 2016; McCracken *et al*., 2014). Organoids, unlike cancer cell lines, preserve all the variables specific to the original IEC, including tumor cells (Wetering *et al.*, 2015). As a result, organoids provide a new and more relevant way to study basic gene functions and cellular processes *in cellulo*. In addition to this, organoid technology also holds great promise for translational research.

1.5. The biology of IL-15: a cytokine that signal via the common γ-chain (γc; CD132) receptor

Cytokines are small cell-signaling protein molecules secreted by different cells that regulate the nature, intensity, and duration of the immune response by exerting a variety of effects on immune cells and other cells. Cytokines form 6 groups according to their functions as innate and adaptive cytokines or as inflammatory and anti-inflammatory cytokines (Cianci *et al.,* 2010). Many interleukins, as well as some growth and hematopoietic factors, are included in type I cytokine group. A common structure with four α-helical bundles is characteristic of this cytokine group (Rochman *et al.,* 2009). Expression of a common γ-chain (γ_c; also known as IL-2Rγ and CD132) defines the IL-2 receptor subfamily, which includes receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (Warren J. Leonard, 2001). IL-2 and IL-15 cytokines share functions that include generation and persistence of NK, stimulation of immunoglobulin synthesis by B cells, generation of cytotoxic T lymphocytes and stimulation of T cell proliferation (Waldmann and Tagaya, 1999).

1.5.1. Structure

IL-15 is a 14-15 kDa glycoprotein of 114 amino acids, mainly produced by macrophages as well as non-lymphoid cells. The gene is located on human chromosome 4q31, and the central region of mouse chromosome 8 (Waldmann, 2006). Characterization of human, murine, and simian IL-15 indicates that this cytokine is conserved between species (73% identity between human and mouse; 97% identity between human and monkey) (Grabstein *et al.*, 1994). Like the IL-2 gene, the human IL-15 gene contains 9 exons (7 coding exons), with a comparable intron/exon structure (Anderson *et al*., 1995). However, homology between IL-2 and IL-15 at the nucleotide or protein level is minimal (Fehniger and Caligiuri, 2001). IL-15 expression is regulated at multiple levels, including transcription, translation, and intracellular trafficking. An increase in IL-15 production predisposes to the risk of abnormal lymphocyte activation and excessive autoreactive T cell survival, leading to the development of autoimmune or chronic inflammatory diseases (Waldmann, 2004).

Compared to the originally discovered IL-15 precursor, with a 48-AA long signal peptide (LSP), an alternative IL-15 precursor protein expresses a 21-AA short signal peptide (SSP). However, an identical mature IL-15 protein in human and mouse is encoded by both IL-15 isoforms (Fehniger and Caligiuri, 2001). LSP-IL-15 is targeted to the secretory pathway (ER/Golgi apparatus), whereas SSP-IL-15 is restricted to the cytoplasm and nucleus, as assessed by IL-15-green fluorescent protein (GFP) fusion protein localization experiments (Gaggero *et al.*, 1999). LSP-IL-15 has been identified in skeletal muscle, placenta, heart, lung, thymus, liver, and kidney, whereas SSP-IL-15 is expressed in heart, thymus, appendix and testis. The biological significance of these different IL-15 isoforms is not clear (Gaggero *et al.*, 1999).

1.5.2. IL-15 receptor

IL-15, as IL-2, recognizes a heterotrimeric receptor composed of three subunits. The alpha subunit is specific for IL-15 or IL-2, while the beta (CD122) and gamma (CD132) subunits are shared between both cytokines (Giri *et al.*, 1995). IL-15Rα and IL-2Rα share structural similarities with a conserved extracellular protein-binding Sushi domain. IL-

15Rα has a 173-amino acid extracellular domain, a single 21-amino acid transmembrane region and a 37-amino acid cytoplasmic domain (Takeshita *et al.*, 1992). The IL-2R/15Rβ receptor comprise a 214-amino acid extracellular segment, a 25-amino acid transmembrane region (Sharon *et al.,* 1986; Tsudo *et al.,* 1987), and a 286-amino acid cytoplasmic domain (Hatakeyama *et al*., 1989). Finally, a 233-amino acid extracellular domain, a 28-amino acid transmembrane domain and an 86-amino acid cytoplasmic region form the human γ_c (Takeshita e*t al*., 1992).

The IL-15Rα (*Il15ra*) gene is located on human chromosome 10 and contains seven exons. Eight different isoforms of IL-15Rα are expressed by alternative splicing (Dubois *et al*., 1999). IL-15 bind IL-15Rα with high-affinity (K_d greater than or equal to 10^{-11} M), but, like IL-2Rα, has no role in signal transduction. Indeed, the IL-2/15Rβ and γ_c are responsible for signal transduction, even if IL-15Rα binds IL-15 with high affinity. Both IL-15 and IL-2, in the absence of IL-15Rα, bind and signal through the heterodimeric IL-2/15Rβ-γc, but with an intermediate affinity (K_d of approximatively 10^{-9} M) (Armitage *et al.,* 1995). This high affinity along with the co-expression of IL-15Rα and IL-15 in the same cell, which allows the intracellular binding of IL-15 to IL-15Rα, leads to expression as a complex on the cell surface. Once on the cell surface, IL-15Rβ-γ_c in an opposing cell can be stimulated by the IL-15Rα/IL-15 complex by cell–cell interactions. This cytokine delivery mechanism, which assumes the formation of an immunological synapse, has been called trans-presentation and is unique in cellular immunology (Fig. 3A) (Dubois *et al*., 2002). This interaction restricts aberrant immune stimulation and decreases the risk of autoimmunity from uncontrolled IL-15 exposure, by limiting the exposure to circulating IL-15. Trans-presentation, considered the major mechanism of IL-15 action *in vivo*, proposes that IL-15Rα may have functions in addition to surface capture. For example, Aaron's group suggested that IL-15Rα may stabilize IL-15 in a conformation that is able to bind IL-2Rβ, similar to the effect of IL-2Rα for IL-2 (Ring *et al.*, 2012). Overall, trans-presentation was proposed as a mechanism to explain how the expression of IL-15Rα by neighbouring cells is critical for IL-15 signalling through $\beta\gamma_c$ (Dubois *et al*., 2002). IL-15 may be also presented by IL-15Rα to $\beta\gamma_c$ in the same cell, in a process

called cis-presentation (Fig. 3B) (Olsen *et al.*, 2007). However, the physiological relevance of this process has not yet been elucidated.

Soluble IL-15Rα (sIL-15Rα) is constitutively generated from the transmembrane receptor through a defined proteolytic cleavage. Existence of IL-15Rα both in membrane bound and in a soluble form, in mice as well as in humans, has been proved by several authors (Vadim Budagian *et al.*, 2004). In fact, it has been shown that a recombinant soluble domain of IL-15Rα could be a selective and potent agonist of IL-15 action, without affecting IL-15 binding and function of the trimeric IL-15Rβ-γ_c (Mortier *et al.*, 2006).

Transcript levels of full-length IL-15Rα are found in numerous tissues and cell lines (Steel *et al.*, 2010). Although the expression of all eight isoforms of IL-15Rα was observed in certain tissues, such as brain, intestine, liver and peripheral blood

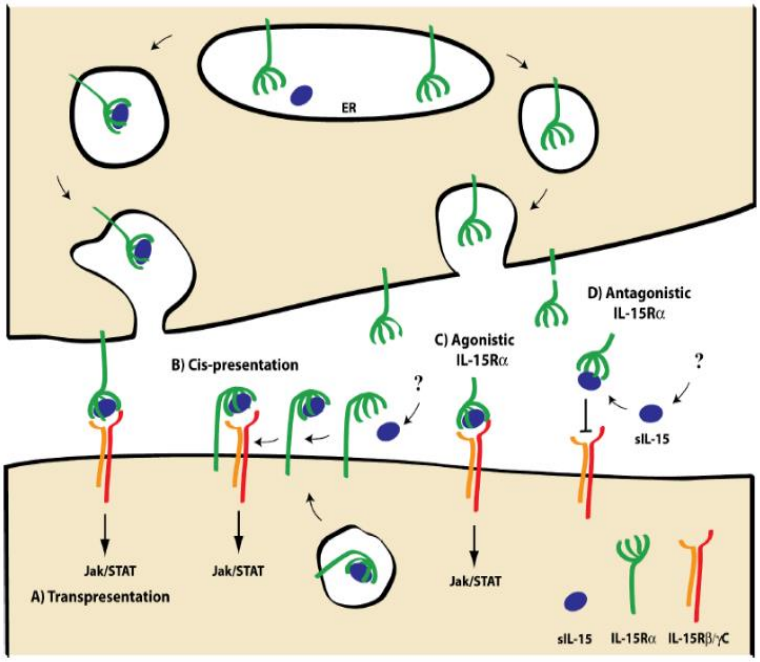

Figure 3. Principal mechanisms for IL-15 delivery (A) Trans-presentation: IL-15Rα and IL-15 are synthetized in the same cell and transported to the cell surface where the cell surface complex can stimulate neighboring cells through the IL-15R βγc. **(B)** Cis-presentation: IL-15 is presented by IL-15Rα on the same cell. These mechanisms may utilize IL-15 derived from autocrine or paracrine sources. **(C)** IL-15/IL-15Rα complexes can be generated artificially and act as agonist to stimulate neighboring cells. **(D)** Cleaved, empty IL-15Rα may act as a sink to bind sIL-15 and antagonize IL-15 activity. (From Stonier and Schluns, 2010, License#4647131264176).

mononuclear cells (PBMC), the relative expression varies for each isoform (Anguille *et al.*, 2009). The availability of free IL-15 may also be negatively affected by the presence of sIL-15Rα in biological fluids, competing with cognate membrane-bound receptors for the cytokine, which constitutes an important protective mechanism against excessive IL-15 activity (Fig. 3C, D) (Budagian *et al*., 2004; Bulfone-Paus *et al*., 2006).

IL-15Rα facilitates proliferation and maintains functions of mature lymphocytes. For example, studies show that *Il15*$^{-/-}$, *Il15ra*$^{-/-}$, *Il2rb*$^{-/-}$ and *Il2rg*$^{-/-}$ mice display deficient numbers of NK, NKT, and TCRγδ IEL (Lodolce *et al.*, 1998). The presentation of membrane-bound IL-15 to IL-2R/15Rβ-γ_c in lymphocytes, activates the JAK and STAT pathway (Beadling *et al.*, 1994). Following cytokine binding, the receptor chains CD 122 and γ_c come together, allowing the activation of associated JAK1 and JAK3. The kinases then phosphorylate the receptor, which leads to the tyrosine phosphorylation of STAT3 and STAT5. Once phosphorylated, STAT3 and STAT5 are translocated to the nucleus as homo- or hetero-dimers, where they bind to target DNA regulatory elements and participate in the activation of gene expression (Leonard, 2001). Additional signaling pathways activated by IL-2 and IL-15 include the phosphorylation of the Src family cytoplasmic tyrosine kinases Fyn, Lck, and Lyn, Syk kinase, phosphatidylinositol 3 kinase (PI3-kinase) and Akt, expression of Bcl-2 anti-apoptotic protein, as well as the Ras/Raf/MEK/mitogen-activated protein kinase (MAPK) pathway (Gu *et al.*, 2000; Miyazaki *et al.*, 1995; X Zhu *et al.*, 1994; Ratthé and Girard, 2004; Uhlin *et al*., 2005).

1.5.3. The pleiotropic role of IL-15 in the intestinal mucosal barrier

In 2001, it was reported that T cells did not require IL-15Rα expression during IL-15 response while IL-15Rα was essential on non-hematopoietic cells (Lodolce *et al.,* 2001). Given the previously shown direct role of IL-15 on T cells, other research was completed in order to clarify the role of IL-15Rα expression in other cell types. The finding that IL-15 was produced by IEC (Reinecker *et al.,* 1996), and that IL-15 was a potent stimulant of IEL (Ebert, 1998), focused the attention of the research community on the possible interaction between enterocytes and IEL mediated by IL-15 (Inagaki-Ohara *et al.,* 1997;

Ohta et al., 2002). IL-15 stimulates IEL and plays a role in T cell activation and recruitment, T cell memory maintenance, stimulation of B cell proliferation and immunoglobulin synthesis, NK cell proliferation, neutrophil activation, and inhibition of apoptosis mediated by the upregulation of anti-apoptotic Bcl-2 family proteins (van Heel, 2006). The levels of anti-apoptotic proteins BCL-2, MCL-1, BCL-xL are increased after IL-15 stimulation (Becker *et al.*, 2002; Lodolce *et al.*, 1998), and pro-apoptotic proteins BAX, BID, BIM, NOXA and PUMA are downregulated (Huntington *et al*., 2009; Van Belle and Grooten, 2005). Activation of NF-kB, as well as inhibition of caspase-3 and -8 can also be some of the mechanisms induced to prevent apoptosis by IL-15 (Hoontrakoon *et al*., 2002; Bouchard *et al.*, 2004). Therefore, IL-15 may be involved in the control of the functional intestinal barrier, based on its pleiotropic role in innate and adaptive immune system.

1.5.3.1. Physiological functions of IL-15 in innate immunity

Monocytes, macrophages and DC effectively transcribe and translate IL-15, and also respond to IL-15 stimulation. IL-15 increases phagocytosis in macrophages, in addition to a pro-inflammatory activity inducing *IL-12*, *IL-8* and *monocyte chemotactic protein-1 (MCP-1)* expression, involved in attracting neutrophils and monocytes respectively to sites of infection (Badolato *et al*., 1997), and secreting IL-6, IL-8 and TNFα (Vadim Budagian *et al.*, 2006). In contrast, low concentrations of IL-15 favor IL-10 production in monocytes. Monocytes and DC have been described as the cell type expressing the highest levels of IL-15Rα, which allows them to trans-present IL-15 (Perera *et al*., 2012). IL-15 is produced by DC and DC differentiation also depends on IL-15 (Regamey *et al.*, 2007). DC incubated with IL-15 display increased expression of CD40, CD83, CD86, and major histocompatibility complex (MHC) class II molecules, in addition to an apoptosis resistant phenotype (Anguille *et al.*, 2009). IL-15 also modulates the adaptive immune response by enhancing the production of IL-2, a major T lymphocyte growth factor (Feau *et al.*, 2005). Unlike T lymphocytes and NK cells, mast cells use an IL-15 receptor without the requirement for the IL-15Rα (Waldmann and Tagaya, 1999). IL-15Rα and $\beta\gamma_c$ are constitutively expressed in human neutrophils (Ratthé and Girard,

2004) protecting them from apoptosis, modulating phagocytosis and the recruitment of other neutrophils to inflammatory sites (Perera *et al.*, 2012). Moreover, neutrophils increase the expression of a high affinity receptor for IgG, CD64, and the receptor for bacterial cell wall lipopolysaccharide CD14 in response to IL-15, increasing their ability to bind to pathogen-specific antibodies and to respond to Gram negative bacterial infections (Abdel-Salam and Ebaid, 2008). γδ T lymphocytes are involved in immunity against malignancies and pathogens (Ismail *et al.*, 2011). These cells, while they are rare in the peripheral blood, are abundant among intestinal IEL (Zhao *et al*., 2005). γδ T cells, unlike classical αβ T cells, recognize non-processed lipid antigens (Tanaka *et al*., 1995). This recognition is not restricted by MHC molecules and is mediated by the TCR. Furthermore, γδT cells have a regulatory effect on neutrophils, DC and B cells in humans, and on neutrophils and macrophages in mice (Casetti *et al.*, 2009). Moreover, during the inflammation at the site of infection, γδ T cells have been shown to reduce the inflammatory reaction and associated tissue damage (Kapp *et al*., 2004).

1.5.3.2. Physiological functions of IL-15 in adaptive immunity

IL-15 plays a critical role in maintaining T lymphocytes, especially memory CD8 T lymphocytes. In *IL-15* and *IL-15ra*-deficient mouse models, it has been demonstrated that IL-15 and IL-15Rα are essential for CD8 T cell survival, by increasing the expression of the anti-apoptotic molecule Bcl-2 (Wu *et al.*, 2002). Recent studies have confirmed that IL-15 trans-presentation by DC is needed for the generation and maintenance of memory CD8 T cells (Stonier and Schluns, 2010), as well as for the enhancement of their effector functions, including those associated with cytolysis and cytokine secretion. In addition, IL-15 modulates several physiological functions, such as proliferation, survival and responsiveness of T cells. For example, mature CD4 T lymphocytes respond to IL-15 depending on the activation status. IL-15 induces a quiescent phenotype in the absence of concomitant TCR triggering, while this quiescence of CD4 T cells is reversed by IL-15 in the presence of concomitant TCR engagement, resulting in increased proliferation (Perera *et al.*, 2012). In addition, IL-15 increases the ability of CD4 T cells to interact with APC, by inducing CD40L (CD154) on previously activated cells (Skov *et al*., 2000).

To maintain FoxP3-expressing $CD4^+CD25^+$ Treg cells and retaining these cells in the periphery, IL-2 is required, while IL-15 has controversial effects on Tregs (Krause *et al.*, 1996). IL-2 plays a critical role in initiating the process of activation-induced cell death (AICD), which leads to the elimination of self-reactive T cells, whereas IL-15 displays anti-apoptotic effects on T cells (Tagaya *et al.*, 1997). In summary, some studies show that IL-15 promotes the generation of induced $FoxP3^+$ Tregs, while others show that IL-15 can also render effector $CD4^+$ or $CD8^+$ cells unresponsive to the regulatory effects of $FoxP3^+$Tregs. Accordingly, proliferation and IFNγ production by $CD4^+$ and $CD8^+$ T cells, in the presence of IL-15, reduce the inhibitory effect of Tregs. While IL-15 does not exert its inhibitory outcome directly on Tregs, IL-15 can disturb the suppressor effects of Tregs on responder T cells (Ahmed *et al.*, 2009). However, Imamichi *et al*. (2008) have found that IL-15 increases CD25 and FoxP3 expression in human peripheral $CD4^+CD25^-$ cells in the absence of antigen stimulation. Thus, despite increasing expression of FoxP3 levels comparable to expression in conventional Treg cells, IL-15-induced $CD4^+$CD25high $FoxP3^+$ cells present only weak suppressor activity.

While B cells numbers are similar in control and mice deficient for IL-15 or IL-15Rα (Budagian *et al*., 2006), some authors still believe that IL-15 can play a minor role on B lymphocytes (Di Sabatino *et al.*, 2011). However, *in vitro*, IL-15 increases immunoglobulin secretion and B lymphocyte proliferation and differentiation (Armitage *et al*., 1995; Budagian *et al*., 2006). Additionally, Fas-mediated apoptosis in B cells can be also prevented by IL-15, as well as the induction of antibody responses partially independent of CD4 (Demirci and Li, 2004; Steel *et al*., 2010). Finally, IL-15 enhances T_H17 cell proliferation in a TCR-dependent (T_H1/T_H17 IFN-γ- and IL-17-producing) manner (Annunziato *et al*., 2007; Pandolfi *et al*., 2009).

1.5.3.3. Role of IL-15 in modulation of enterocytes

As we have seen above, IL-15 has a potent role on the survival and immunomodulatory effects on innate and adaptive immune cells, as well as in the defence mechanisms against pathogens, especially in the gut (Perera *et al.*, 2012). In addition, in the active phase of some gastrointestinal inflammatory pathologies, there is an increased expression of IL-

15 (Kirman and Nielsen, 1996; Vainer *et al*., 2000). Two different groups have shown apparently conflicting conclusions on the role of IL-15 in IBD, with the mouse model of colitis induced by DSS. On one hand, Obermeier *et al*. (2006) have shown that blocking IL-15 using soluble IL-15Rα resulted in the exacerbation of the disease in a chronic DSS-induced colitis model (Obermeier *et al.*, 2006). In this study, IL-15 neutralization reduced inflammatory infiltration and pro-inflammatory cytokine production. An anti-apoptotic effect of Fas-induced apoptosis by IL-15 on IEC, both *in vitro* and *in vivo*, was observed. In their model, they argue that IL-15 could play not only a role as a pro-inflammatory cytokine, but also could have the potential to reduce mucosal damage by preventing IEC apoptosis. On the other hand, Yoshihara *et al*. (2006) demonstrated in an acute DSS-induced colitis model that *Il15*$^{-/-}$ mice were protected against DSS-induced colitis (Yoshihara *et al*., 2006). In these IL-15-deficient mice, the numbers of NK and CD8^{+} T cell populations in the lamina propria were reduced. Levels of IFNγ, TNFα and IL-12p40 detected in culture supernatants of LP cells were reduced, indicating that IL-15 may act as a pro-inflammatory cytokine contributing to DSS-induced colitis pathogenesis. Obermeier *et al*. explain the divergence between these results by arguing that in IL-15 null mice, the protective functions of IL-15 on IEC can be compensated by other factors through different intestinal stage development. In Yoshihara *et al*. (2006), the genetic deletion of IL-15 occurred in utero before adult colitis induction, whereas antibody neutralization of IL-15 occurred later in development in the Obermeier *et al*. study. Despite these apparently contradictory data on the functional role of IL-15 in these two models, it is certain that IL-15 is up-regulated in the inflamed gut mucosa.

1.5.4. IL-15 in inflammatory bowel diseases

Increasing evidence indicates that IBD is characterized by infiltration of macrophages, activated T and B cells, which secrete massive amounts of cytokines, enhancing intestinal mucosal inflammation (Cianci *et al.*, 2010; Di Sabatino *et al.*, 2011; Pandolfi *et al.*, 2009). Altered patterns of proinflammatory cytokine production by innate and adaptive immune cells from the periphery and the lamina propria play a crucial role in the pathogenesis of both experimental colitis and IBD (Fuss *et al.*, 1996).

As we mentioned earlier, abnormal expression of cytokines, including IL-15, is observed in IBD. However, in a transgenic mouse model, IL-15 overproduction resulted in the development of fatal leukemias (Fehniger and Caligiuri, 2001), suggesting that overproduction of IL-15 could exert potentially harmful effects to the host. Indeed, elevated IL-15 levels have been found in several autoimmune and inflammatory diseases, such as rheumatoid arthritis (McInnes and Schett, 2007), multiple sclerosis (Rentzos *et al.*, 2005), celiac disease (Mention *et al.*, 2003) and IBD (Nishiwaki *et al.*, 2005). The number of IL-15 expressing cells is elevated in the rectal mucosa of IBD patients with active UC (Kirman and Nielsen, 1996). IL-15 activity is increased, even in inactive UC (Sakai *et al.*, 1998). Additionally, overexpressing human IL-15 preferentially in IEC in a transgenic mouse model increases the number of $CD8^+$ T cells, and IFNγ and TNFα production by NK cells in the LP, correlating with enhanced chronic inflammation of the small intestine (Ohta *et al.*, 2002). These data suggest that IL-15 could be relevant to the pathology of IBD, by preferentially expanding and activating $CD8^+$ T cells in the small intestine of mice, correlating with mucosal damage characteristic of IBD.

IL-15-activated lamina propria T cells from patients with IBD induce the production of IL-12 in monocytes-macrophages, and IFNγ secretion by activated T cells, leading to a T_H1 phenotype and enhancing the activity of NK cells and $CD8^+$ T cells. Then, IL-12/ IFNγ secretion linked to IL-15, together with increased IL-15 mediated expression of CD40L on T cells, recruit leukocytes and induce monocyte and T cell activation, and enhance pro-inflammatory cytokine production, which are believed to be the principal cause of tissue damage and chronic inflammation (Di Sabatino *et al.*, 2011).

Treatment of CD patients with Infliximab, a chimeric monoclonal anti-TNFα, led to decreased serum IL-15, as well as increased sIL-15Rα and IL-15/sIL-15Rα complex levels in patients who responded to Infliximab, in contrast to non-responder patients (Bouchaud *et al.*, 2010). However, in a more recent study, no change in the release of serum sIL-15Rα was observed in IBD patients treated with Infliximab (Perrier *et al.*, 2013). Therefore, the hypothesis that sIL-15Rα limits IL-15 activity needs further experimental confirmation. In addition to this, the IL-15 inflammatory pathway can be a

possible target of new therapeutic approaches in IBD. Due to its pro-inflammatory activities and anti-apoptotic effects, IL-15 has been postulated to have a key role in the development of several autoimmune and inflammatory diseases, such as Celiac Disease and IBD. Moreover, IL-15 may also contribute in the pathogenesis of lymphomagenesis, mediating the development of oligo-monoclonal T cell proliferation, and leading to type II Refractory Celiac Disease (RCD) and Enteropathy Associated T-cell Lymphoma (EATL) (Kagnoff, 2007). Thus, IL-15 could be considered as a strategic target of new biological therapies of some diseases. Such therapies are now becoming possible with the advent of monoclonal anti-IL-15 antibodies (HuMax-IL15, Genmab), blocking the epitope of IL-15 binding to the γ_c subunit of the IL- 15 receptor (Baslund *et al.*, 2005), and pharmacologic agents who can selectively block IL-15 signal transduction pathways (Kagnoff, 2007).

1.6. Thesis premises

The main forms of IBD, CD and UC, are chronic relapsing disorders of the gastrointestinal tract that are characterized by pathological intestinal inflammation and epithelial injury (Baumgart and Sandborn, 2012; Danese and Fiocchi, 2011). These disorders are related with marked morbidity and can have a major impact on an individual's quality of life. Recent genetic and immunological studies have uncovered the primary role of cytokines and cytokine signaling in IBD, by controlling intestinal inflammation and the associated clinical symptoms (Strober *et al.*, 2002). A combination of several risk factors initiates epithelial barrier function alterations, leading to translocation of luminal antigens into the bowel wall. Then, excessive cytokine responses cause subclinical or acute mucosal inflammation. The failure to resolve this acute intestinal inflammation in immune-predisposed patients leads to the development of chronic intestinal inflammation and uncontrolled activation of the mucosal immune system. Mucosal immune cells, such as T cells, macrophages and ILC, respond to microbial products or antigens from the commensal microbiota by expressing cytokines that can promote chronic inflammation on the gut. Additionally, cytokines are associated with IBD complications such as intestinal stenosis, rectal bleeding, abscess and fistula formation, and the development of colitis-associated neoplasias (Anderson *et al*., 2011; Baumgart and Sandborn, 2012; Becker *et al*., 2004). IL-15 is a cytokine with potent immunomodulatory effects on immune cells and may be involved in the pathogenesis of both animal experimental colitis and IBD. As immune cells are implicated in perpetuation of inflammation in IBD, and as the role of IL-15 in the control of intestinal mucosa inflammation is unclear, I have addressed these issues by using *in vivo* mouse models, as well as *in vitro* models under conditions representative of pathological inflammation.

1.7. Hypothesis

Based on the above premises, I hypothesize that ***IL-15 signaling by intestinal epithelial cells promotes the development of colitis by inducing gut inflammation.*** I hypothesize that IL-15 pathway inhibition may reduce colitis symptoms.

1.8. Objectives

The specific aims of my research project are:

1. **To evaluate the role of IL-15 in intestinal homeostasis and the development of colitis.**

a. Characterize the intestinal mucosal phenotype of *Il15*$^{-/-}$ mice by macroscopic and microscopic analysis.

b. Evaluate the severity of colitis in WT and *Il15*$^{-/-}$ mice during the induction of colitis after DSS treatment.

2. **To determine the potential therapeutic efficacy of the anti-IL-15Rβ antibody in colitis.**

a. Evaluate the severity of tissue damage in WT and *Il15ra*$^{-/-}$ DSS-treated mice following inhibition of IL-15 signaling with anti-IL-15Rβ.

3. **To investigate the influence of epithelial IL-15 on intestinal proliferation and repair.**

a. Determine the role of autocrine IL-15 signaling in specific IEC differentiation and inflammatory responses on small intestinal enteroids from crypts isolated from wild-type (WT) and *Il15*-deficient mice.

2. MATERIALS AND METHODS

2.1. Mice

Mice were maintained in filter-topped cages in conventional facilities and fed with standard chow diet and water unless specified otherwise. All experiments were carried out with the approval of the institutional ethics committee by the Animal Care Committee of the Faculty of Medicine and Health Sciences of the Université de Sherbrooke (AEC approval number FMSS-397-15 and 196). Wild type (WT) C57Bl/6 mice were obtained from Charles River. *Il15*-deficient C57Bl/6 (*Il15*$^{-/-}$) mice on C57Bl/6 background were purchased from Taconic and have been backcrossed to C57Bl/6 mice in our colony (Ramanathan *et al*., 2006). *Il15ra*$^{-/-}$ mice obtained from the Jackson Laboratory were backcrossed to C57Bl/6 mice (Bobbala *et al*., 2017).

NOD (NOD/ShiLtJ) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). NOD *Il15*$^{+/-}$ progeny from the 11th backcross onwards was intercrossed to generate NOD *Il15*$^{-/-}$ mice and NOD *Il15*$^{+/+}$ littermates (Bobbala *et al*., 2012).

2.2. Induction of colitis in mice

To induce acute colitis, 3-month-old WT and *Il15*$^{-/-}$ mice drank a solution of DSS 3% (wt/vol, molecular weight 36-50 kDa; ICN Biochemicals, Aurora, OH) dissolved in drinking water, as opposed to control mice with only drinking water. These experiments were performed in both male and female mice (WT H_2O, *Il15*$^{-/-}$ H_20, WT DSS, *Il15*$^{-/-}$ DSS). Mice were maintained on 3% DSS containing water or just water for 7 days before sacrifice. Body weight was monitored every day. When the weight decreased by 20%, mice were euthanized.

2.3. Preventive treatment with the TM-β1 antibody against the IL-15 receptor β-chain

WT and *Il15ra*$^{-/-}$ mice were injected intraperitoneally with 200 µg of purified TM-β1 monoclonal antibody (mAb) (Tanaka *et al*., 1993), or with PBS as control, once on the first day of the one-week DSS 3% treatment (WT TM-β1 H_2O, WT PBS 3% DSS, WT Tm-β1 3% DSS, *Il15ra*$^{-/-}$ TM-β1 H_2O, *Il15ra*$^{-/-}$ PBS 3% DSS , *Il15ra*$^{-/-}$ TM-β1 3% DSS).

Body weight was monitored every day. Mice were killed after 7 days, the intestine and colon were fixed for histological analysis, and colon length was measured.

2.4. Inflammation and histological scores

The inflammatory histological score was determined on histological sections of the colon. The scores shown in Table 1 are a reflection of the inflammation present in the intestinal mucosa and take into consideration the damage to the crypts as well as the extent and severity of the inflammation (Dieleman *et al.*, 1996).

Table 1. Inflammatory histological score

Criteria	***Rating***	***Observation***
Crypt damage	*0*	*None*
	1	*First 1/3 of the low reaches*
	2	*2/3 of the bottom reaches*
	3	*Intact surface epithelium*
	4	*No healthy epithelium*
Extent of inflammation	*0*	*None*
	1	*Restricted to the mucosa*
	2	*Mucosa and submucosa affected*
	3	*Transmural Inflammation*
Severity of inflammation	*0*	*None*
	1	*Light (<25% of the field)*
	2	*Moderate (25-50% of the field)*
	3	*Severe (50 to 100% of the field)*

2.5. Histological procedures

2.5.1. Fixing

Longitudinal sections of large and small intestines were fixed overnight at 4°C in paraformaldehyde (PFA)/PBS 4% solution (Gibco, Invitrogen, CA, USA) to retain the antigenicity of the target molecules and preserve tissue morphology. The solution of 4% PFA was then replaced with 70% ethanol. To assess mucus in the intestine, the large intestine was fixed in Carnoy's solution (100% ethanol, chloroform and glacial acetic acid in a 6:3:1 ratio) for 2 hours at 4°C (Matsuo *et al*., 1997). Carnoy's solution was then replaced with 100% ethanol before cross-sections were included in paraffin. The different tissues were then dehydrated, included in paraffin at the Histology and Electron microscopy Facility of the FMSS, and then cut to 5-µm thick sections before being deposited on charged slides (Thermo Fisher Scientific).

2.5.2. Hematoxylin-Eosin staining

To visualize the general tissue architecture, Hematoxylin & Eosin (H&E) biochromatic staining was performed. Intestinal sections were de-paraffinized in xylene and rehydrated in serial dilutions of ethanol and water before staining. Slides were stained with Harris's hematoxylin solution to visualize negatively charged basophilic structures such as the nucleus. After 5 minutes, slides were washed in water and fixed in acid-water solution (1% acetic acid), followed by rinsing in saturated aqueous lithium carbonate, and staining with eosin for one minute to label the acidophilic structures found in the cytoplasm. Finally, slides were washed in 50% ethanol and dehydrated in increasing percentages of ethanol (100%, 90%, 80%) solutions and xylene. At the end of the procedure, samples were coverslipped using Permount™ Mounting Medium (SP15-500, Fisher Scientific, Waltham, MA, USA). Slides were scanned with Nanozoomer slide scanner (Hamamatsu, Japan) to visualize the complete section in a single microscopic field.

2.5.3. Alcian blue staining

Alcian blue staining permits the visualization of acidic mucins produced by intestinal goblet cells. Alcian blue staining was performed on cross-sections fixed in Carnoy's

solution by the Histology and Electron microscopy Facility of the FMSS, before scanning with the Nanozoomer digital scanner. The entire mucus thickness was measured under a NDP.view2 Plus Image viewing software (U12388-02). The average thickness was calculated by measuring at least ten different spots in a 300 μm distal colon region per mouse in a blind fashion.

2.6. Enteroid cultures

2.6.1. Noggin-Fc and R-spondin 1-Fc conditioned media production

293T cells used for Noggin-Fc and R-spondin 1-Fc production were kindly provided by Dr. Dana Philpott (University of Toronto). Cells were cultured in T75 cm^2 flasks in DMEM (Gibco) supplemented with 10% FBS, GlutaMAX (2 mM), HEPES (10 mM) (Wisent, Quebec, Canada), penicillin/streptomycin (100 U/mL). Puromycin (10 mg/mL) (Wisent) was used for selection of Noggin-Fc producing cells or Zeocin (10 mg/mL) (Invitrogen) for selection of R-spondin 1-Fc producing cells. Selection was stopped and cells were split in T175 cm^2 flasks in Advanced DMEM/F12 (Gibco) supplemented with GlutaMAX (2 mM), HEPES (10 mM), penicillin/streptomycin (100 U/mL). After one week of culture, the medium was collected and centrifuged at 150 x g, for 5 minutes. Supernatants were filtered with 0.22 μm membranes and stored at -80°C. Expression of R-spondin or Noggin was quantified by spot blots to detect the Fc portion with anti-mouse and anti-human Fc secondary antibody, respectively, coupled to HRP (GE Healthcare Life Sciences).

2.6.2. Enteroid culture

After sacrifice, murine jejuneum were collected, opened longitudinally and rinsed with cold PBS. The intestine was cut into small pieces of about 5 mm length and fragments were washed twice by stirring them in a 50 ml Falcon tube containing cold PBS. Intestinal pieces were transferred in 20 mL of PBS containing 30 mM EDTA in a 50 mL tube (Falcon) for 20 minutes on ice, before replacing the EDTA solution with 40 mL of PBS. Subsequently, the tube was vigorously shaken for 5 to 8 minutes at room temperature until a good number of crypts was visualized under the microscope. Next, the suspension was filtered through a 70 μm cell strainer to purify the crypts and to remove residual villi.

Crypts in the eluate were sedimented twice by centrifugation at 150 x g, for 5 minutes at 4˚C, and the pellet was washed twice with 25 mL of DMEM-F12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12) containing Glutamax (2 mM), HEPES (10 mM), penicillin/streptomycin (100 U/mL). The pellet, which contained the crypts, was resuspended in 200-300 µL of Matrigel (Corning) avoiding air bubbles, and 20 µl of the Matrigel-crypt mixture was deposited at the center of each well of a 48-well plate (Sarstedt). The plate was incubated at 37°C for at least 15 minutes until the Matrigel was completely solidified. Medium containing Advanced-DMEM-F12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12) with Glutamax (2 mM), HEPES (10 mM), penicillin/streptomycin (100 U/mL), 1,25 mM N-acetylcysteine (Sigma), 50 ng/mL EGF (Life Technologies), 2% B27 supplement (Gibco), 1% N2 supplement (Gibco), 10% Advanced DMEM/F-12 Noggin medium, and 20% Advanced DMEM/F-12 R-Spondin 1 medium, was added to the wells.

2.6.3. Enteroid treatment

mIFNγ and mIL-15 (Peprotech) were reconstituted according to the manufacturer's instructions. IFNγ and IL-15 were stored at -80˚C until used. Enteroids were passaged and stimulated with mIFNγ (10 ng/ml) or mIL-15 (10 ng/ml) for 48 hours. Pictures were taken every 24 hours. After 3 days of culture, the enteroid-Matrigel mixture was suspended in 300 µL/well Cell Recovery Solution for one hour at 4ºC, to dissolve the Matrigel. Enteroids were then separated by gently mixing up and down, and transferred to low bind tubes for further centrifugation at 150 x g for 3 minutes at 4°C. The supernatant was removed slowly, and the pellet resuspended in TRIzol® (Life Technologies) for RNA extraction. For inclusion in paraffin, after removal of the Cell Recovery Solution, enteroids were washed twice with cold PBS. Next, the pellet was resuspended in PFA 4% for one hour at 4°C and then in 70% ethanol for one hour at 4°C. Subsequently, Histogel (Thermofisher) was used to place enteroids in cassettes, before sending to the Histology and Electron microscopy Facility of the FMSS for inclusion in paraffin.

2.7. RNA isolation and reverse transcription

Enteroids were directly lysed in 1 ml of TRIzol®. Samples were homogenized with chloroform and centrifuged for 15 minutes at 12000 x g, to separate the two phases. The aqueous phase was collected in a new tube and isopropanol was added to precipitate RNAs, for 30 minutes, before a 10-minute centrifugation at 12000 x g. Then, the RNA pellet was washed in 75% ethanol and suspended in RNAse-free water. Purity of the extracted RNA was evaluated by measuring the 260/280 nm and 260/230 nm absorption ratios with Nanodrop 2000 (Thermo Fisher Scientific). One µg of RNA was run on a denaturing formaldehyde-agarose gel to assess RNA quality.

2.8. Quantitative real-time RT-PCR

cDNAs were synthesized from 1 µg total RNA using Quantitect® (Qiagen). Primers were selected by analyzing the efficiency and melting curve for each primer. Murine primer sequences are listed in Table 2. Gene expression was evaluated with the MyQi5® cycler (Bio-Rad) using SYBR Green Supermix (Bio-Rad). Relative expression of the various gene targets was calculated by comparing to the expression of the mouse ribosomal gene 36B4 reference gene. Untreated/non-stimulated enteroids served as experimental controls.

Table 2. List of murine primer sequences

Gene	*Sense*	*Anti-sense*
mSis	*5'-TCAAGAAATCACAACATTCAATTTACTAG-3'*	*5'-CTAAAACTTTCTTTGACATTTGAGCAA-3'*
mLgr5	*5'-CGAGCCTTACAGAGCCTGATACC-3'*	*5'-TTGCCGTCGTCTTTATTCCATTGG-3'*
mMuc2	*5'-GGCCTCACCACCAAGCGTCC-3'*	*5'-TGGGCTGGCAGGTGGGTTCT-3'*
mChgb	*5'-CCAGAGCCAGGAAGAATC-3'*	*5'-CTTCGTAAGAGGACTTGTTG-3'*
mReg3b	*5'-CAGACCTGGTTTGATGCAGA-3'*	*5'-AATTCGGGATGTTTGCTGTC-3'*
mIl15	*5'-CCTTAAGAACACAGAAACCCATG-3'*	*5'-AGGAAACACAAGTAGCACGAG-3'*
mIl15ra	*5'-TCTCCCCACAGTTCCAAAATG-3'*	*5'-TGATTTGATGTACCAGGCCAG-3'*
mIl6	*5'-AGTCCGGAGAGGAGACTTCA-3'*	*5'-TTGCCATTGCACAACTCTTT-3'*
mCxcl10	*5'-CCAAGTGCTGCCGTCATTTTC-3'*	*5'-GGCTCGCAGGGATGATTTCAA-3'*
mTnfa	*5'-CGTCGTAGCAAACCACCAAG-3'*	*5'-GAGATAGCAAATCGGCTGACG-3'*
m36b4	*5'-TCTGGAGGGTGTCCGCAAC-3'*	*5'-CTTGACCTTTTCAGTAAGTGG-3'*

2.9. Statistical analysis

Statistical analysis was performed with the GraphPad Prism 6 Software (San Diego, USA). The statistical significance (*p* value) was calculated by non-parametric comparison between two groups (Mann Whitney test) or one-way analysis of variance test (ANOVA) between the means of more than two independent groups. Differences were considered significant at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

3. RESULTS

3.1. *Il15* deficient mice display normal intestinal epithelium

In order to evaluate the contribution of IL-15 to intestinal homeostasis, several macro- and microscopic parameters were systematically measured in 3- to 5-month old WT and *Il15*$^{-/-}$ knockout mice. Body weight, colon length, as well as the architecture of the colon was measured in tissue samples.

3.1.1. *Il15*$^{-/-}$ mice show no clinical signs of intestinal inflammation

Mice with spontaneous or induced colitis often show weight loss and narrowing of the small intestine or colon (Kim *et al.*, 2012). However, mice lacking *Il15* did not display basal differences in body weight and colon length when compared to controls (Fig. 5).

3.1.2. Intestinal epithelium architecture is preserved despite *Il15* deficiency

As *Il15* deficiency results in a reduction in populations of intra-intestinal epithelial cells present in the epithelial lining of the intestine (Lodolce *et al.*, 1998), we assessed whether any damage to the epithelium was observed at the microscopic level. At first glance, loss of *Il15* does not appear to cause intestinal inflammation. Histopathological analysis of H&E stained sections, that allows the visualization of the structure of a tissue by differentially dyeing the nucleus and cytoplasm, shows that the overall architecture of the intestine is not affected in *Il15*-deficient mice (Fig. 4A). Crypt to surface epithelium lengths of the colon were also measured since inflammation can lead to elongation of these structures (Erben *et al*., 2014). Lengths in the proximal and distal colon did not show any differences (Fig. 4B). Therefore, our results demonstrate that the loss of *Il15* in the intestinal epithelium does not cause overt basal changes in intestinal inflammation at homeostasis.

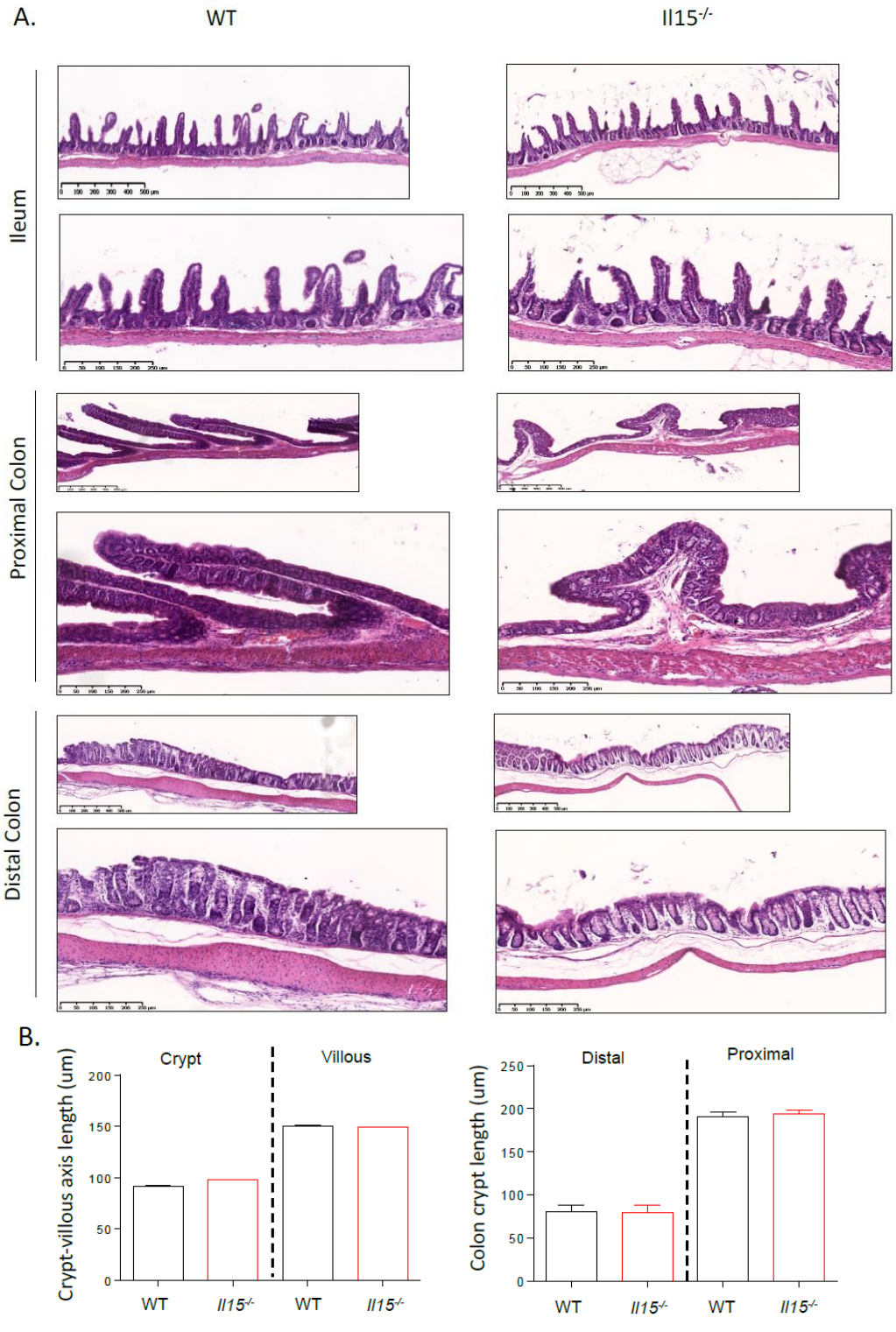

Figure 4. *Il15*$^{-/-}$ mice exhibit normal intestinal epithelial architecture. **(A)** Sections of ileum, proximal and distal colon collected from WT and *Il15* deficient mice were stained with H&E. Representative images from at least 4 mice for each group are shown. Magnification 5X (Scale bar represents 500 µm) and 10X (Scale bar represents 250 µm). **(B)** The crypt-villus (ileum) and the crypt to surface epithelium (colon) length was measured in WT and *Il15* deficient mice (WT n = 4, *Il15* $^{-/-}$ n = 4).

3.2. WT and *Il15* deficient mice display equal susceptibility to DSS-induced colitis

Given the role of inflammatory cytokines in the development of IBD, we wanted to evaluate the role of IL-15 in a model of induced colitis. To this end, 3-6 month-age-matched WT and *Il15*$^{-/-}$ male mice were treated with either H_2O or DSS 3% for one week.

3.2.1. Weight loss and colon length are comparable in WT and *Il15*-deficient mice after DSS treatment

Mice were monitored for macroscopic manifestations related to DSS-induced colitis (Fig. 5). Both groups of mice lost weight in a comparable manner during DSS treatment. Untreated WT and *Il15*$^{-/-}$ mice showed no significant differences in body weight during the experimental window (Fig. 5A). Similarly, loss of colon length was comparable between WT and *Il15*$^{-/-}$ mice treated with DSS (Fig. 5B). Fig. 5C shows colons from WT and *Il15*-deficient control mice treated with H_20 and WT and *Il15*-deficient mouse treated with DSS for one week. These observations suggest that absence of *Il15* does not influence colon length or weight loss following DSS treatment.

3.1. DSS-induced intestinal damage is comparable between WT and *Il15*$^{-/-}$ mice

The above results suggest that *Il15*-deficient mice are equally susceptible to DSS-induced colitis. Histological analysis of ileum, proximal and distal colon revealed the presence of equivalent intestinal inflammation in DSS-treated WT and *Il15*-deficient mice (Fig. 6A, 6B). The ileum of both DSS treated groups showed no signs of inflammation. The proximal colon exhibited restricted areas of inflamed tissue despite the preservation of the overall architecture. In the distal colon, regions of lower inflammation were interspersed with severe areas of inflammation where the epithelium was completely eroded.

3.1.1. Mucus production is equally perturbed in the colon of DSS-treated WT and *Il15*$^{-/-}$ mice

To determine whether absence of *Il15* affects the production of mucus, we evaluated the expression of mucus by Alcian blue staining in the colon from control and DSS-treated mice. Due to the complete erosion of the epithelial layer that contains the goblet cells in

the distal epithelium, the amount of mucus present in the lumen was diminished in both groups of DSS-treated mice (Fig. 7). These results indicate that the absence of *Il15* does not influence the level of mucus production nor the level of goblet cell destruction, when compared to WT mice.

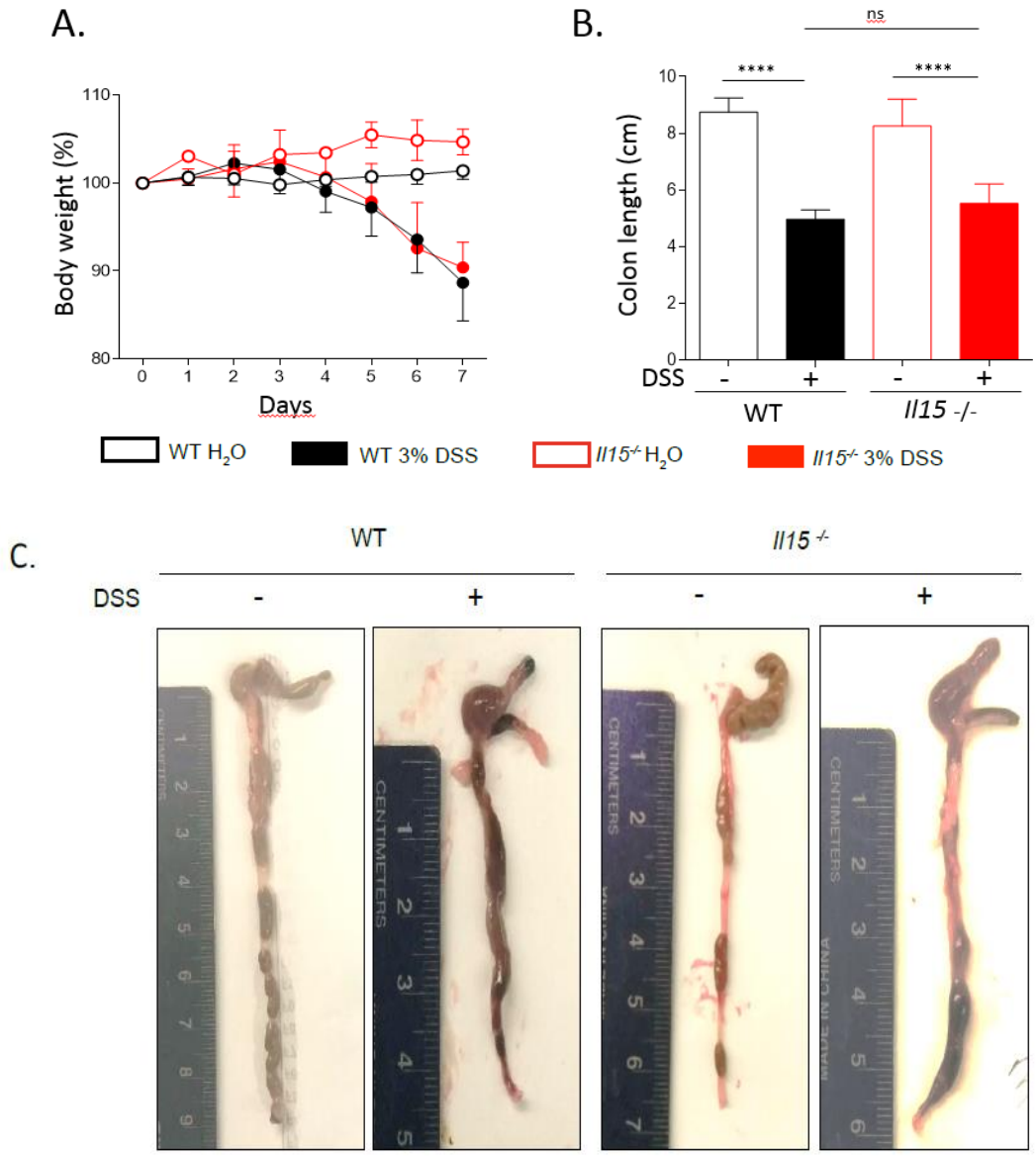

Figure 5. WT and *Il15*$^{-/-}$ mice experience a drastic weight loss and show macroscopic signs of intestinal inflammation after DSS treatment. (A) Body weight measurement. **(B)** Colon length at sacrifice. **(C)** Representative images of colon from the four groups are shown. WT H_2O n=4, *Il15*$^{-/-}$ H_2O n=4, WT DSS n=8, *Il15*$^{-/-}$ DSS n=8). Error bars represent the standard error of the average. Statistical significance using one-way ANOVA test is shown by * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.

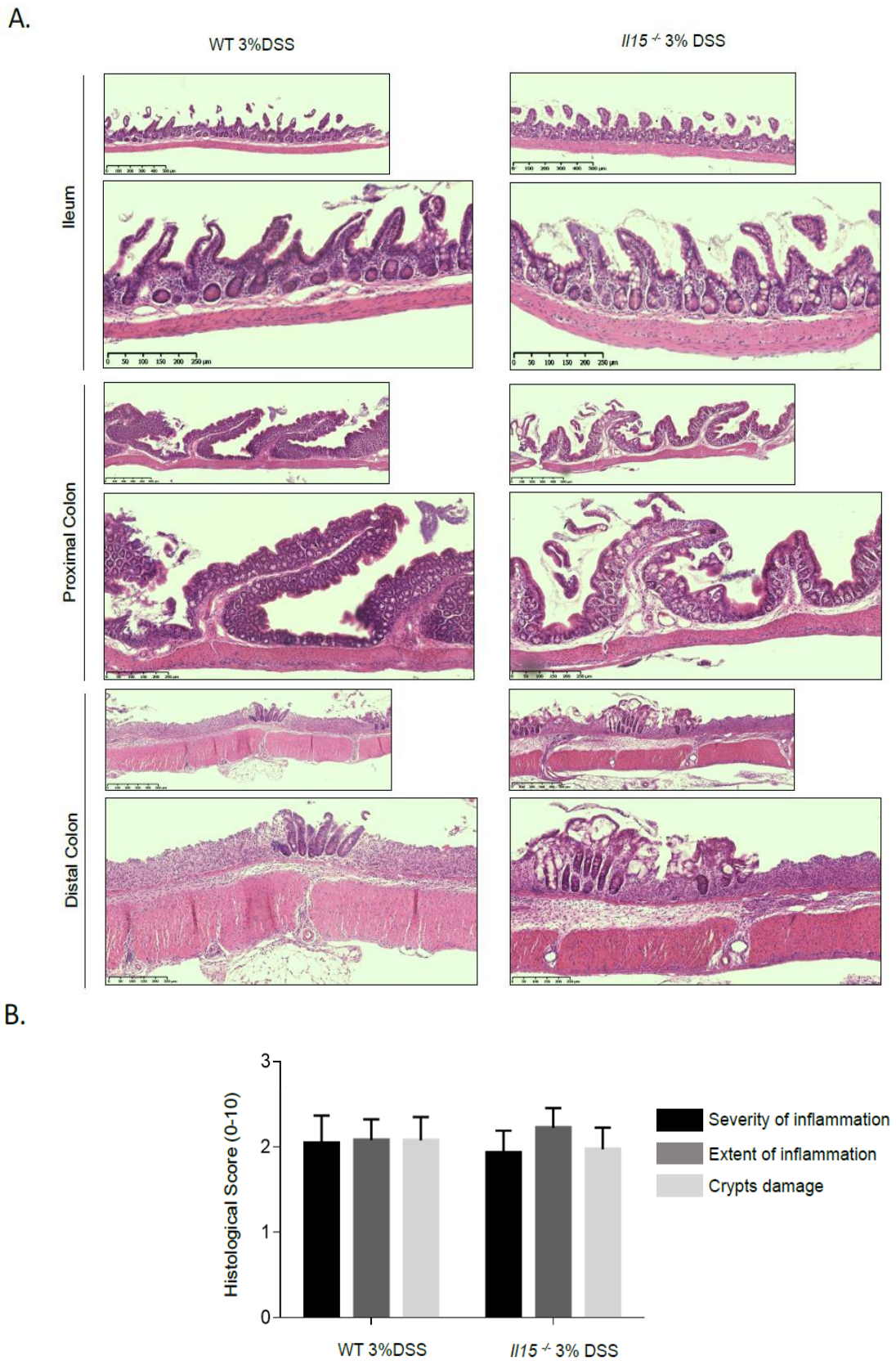

Figure 6. WT and *Il15*$^{-/-}$ mice show severe colitis after DSS treatment. **(A)** Sections of ileum, proximal and distal colon collected from WT and *Il15*$^{-/-}$ DSS-treated mice were stained with H&E. Representative images from at least 4 mice for each group are shown. Magnification 5X (Scale bar represents 500 µm) and 10X (Scale bar represents 250 µm). **(B)** Histological analysis of colonic sections stained with H&E reveals colitis following a proximal to distal severity gradient in WT and *Il15* deficient mice. (WT DSS n=8, *Il15*$^{-/-}$ DSS n=8) Error bars represent the standard error of the average. Statistical significance using Mann Whitney test showed no significant differences.

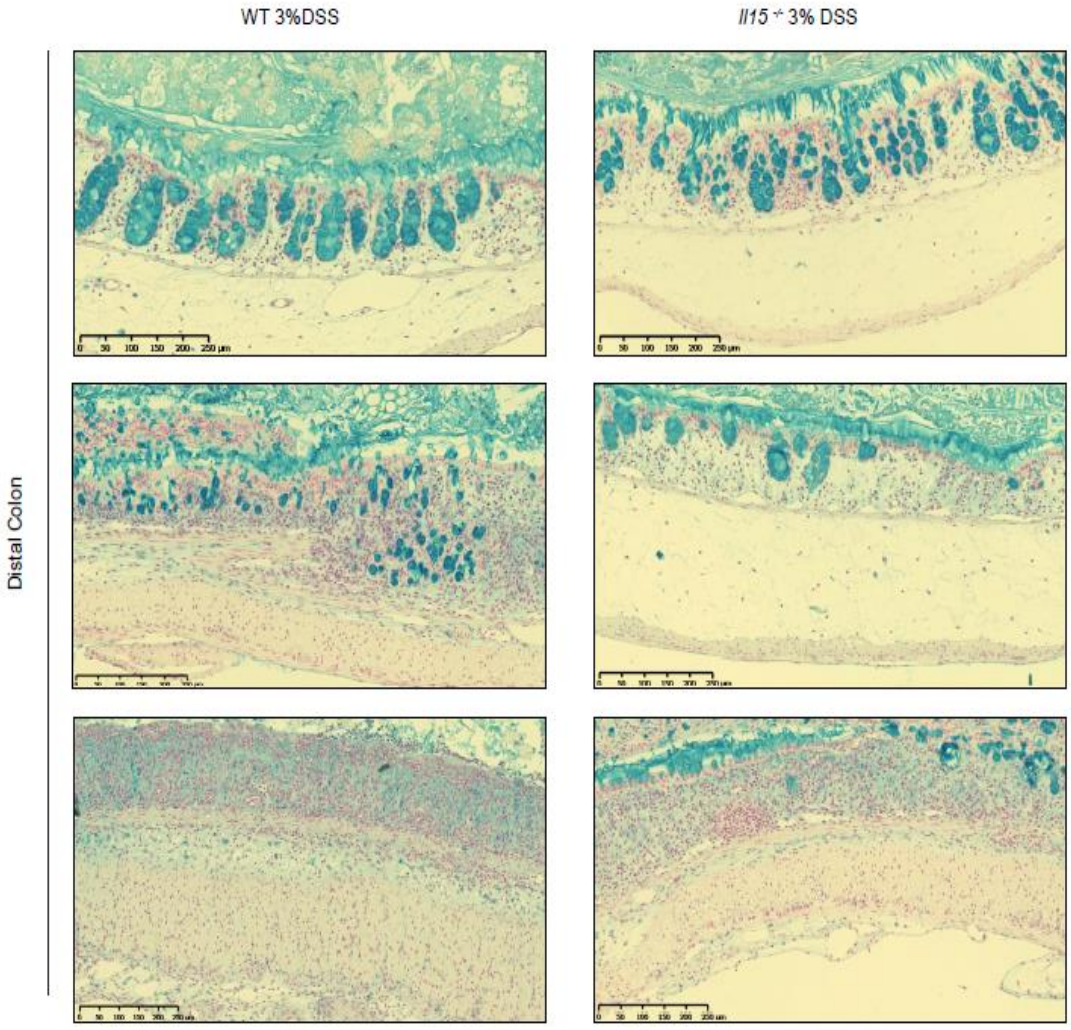

Figure 7. WT and *Il15*$^{-/-}$ mice show an equal reduction in the number of colonic goblet cells and mucus production after DSS treatment. Goblet cells and mucins were stained with Alcian blue, on paraffin embedded distal colon sections of DSS treated *Il15* deficient and WT control mice**.** Representative images from at least 4 mice for each group are shown. Magnification 10X (Scale bar represents 250 µm). (WT n = 8, *Il15*$^{-/-}$ n = 8).

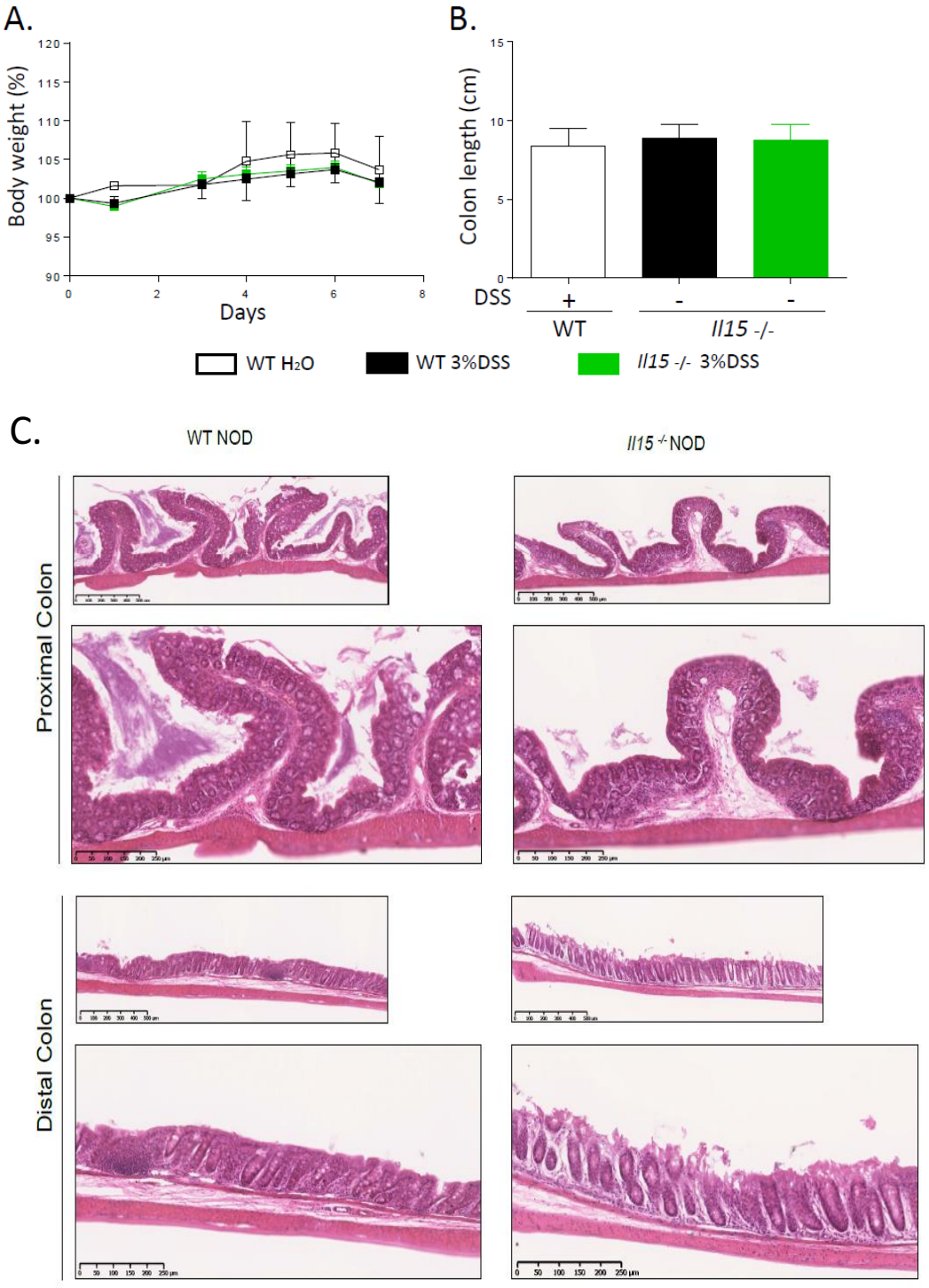

Figure 8. NOD mice are resistant to DSS-induced colitis. (A) Body weight was measured every day during DSS treatment. **(B)** The length of the colon was determined at sacrifice. **(C)** Sections of proximal and distal colon from the WT and *Il15* deficient DSS-treated NOD mice were stained with H&E. Magnification 5X (Scale bar represents 500 µm) and 10X (Scale bar represents 250 µm). (NOD WT H_2O n=3, NOD WT 3% DSS n=4, NOD, *Il15*$^{-/-}$ 3% DSS n=4). Error bars represent the standard error of the average, Statistical significance using one-way ANOVA test is shown by * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.

3.2. *Il15* deficiency does not influence the susceptibility of NOD mice to DSS-induced colitis

One of the major scientific challenges in exploring the etiology of complex inflammatory diseases is finding a good animal model. Other alternative models for the study of ulcerative colitis using mice with a NOD background have already been studied (Palamides *et al.*, 2016). *Il15*-deficient NOD mice were not susceptible to autoimmune type 1 diabetes (Bobbala *et al.*, 2012). We induced DSS-mediated colitis in NOD and *Il15*$^{-/-}$ NOD mice as described above, to determine whether the absence of *Il15* could alter the response of NOD mice to DSS-induced colitis.

3.2.1. NOD mice are not susceptible to DSS-induced colitis

To determine whether *Il15* deficiency influenced the development of colitis in the NOD background, we treated NOD and *Il15*$^{-/-}$ NOD mice with 3% DSS. Unlike C57Bl/6 mice, NOD and *Il15*$^{-/-}$ NOD mice did not show any loss of body weight during DSS treatment (Fig. 8A). At sacrifice, colon length was comparable to that of untreated controls (Fig. 8B).

3.2.2. The epithelial architecture is conserved in the middle colon of NOD mice under DSS treatment

Histological analysis showed that the epithelial architecture was conserved in most of the intestinal tissue (Fig. 8C), as reflected by the absence of inflammatory histological scores (data not shown). In addition, colonic crypt to surface epithelium length was not significantly modulated in NOD and *Il15*$^{-/-}$ NOD mice by DSS treatment. Collectively, the above results show the absence of inflammation in NOD mice, and that *Il15* deficiency does not alter the phenotype. These results are similar to the reports showing that NOD mice are resistant to DSS-induced colitis (Mähler *et al.*, 1998).

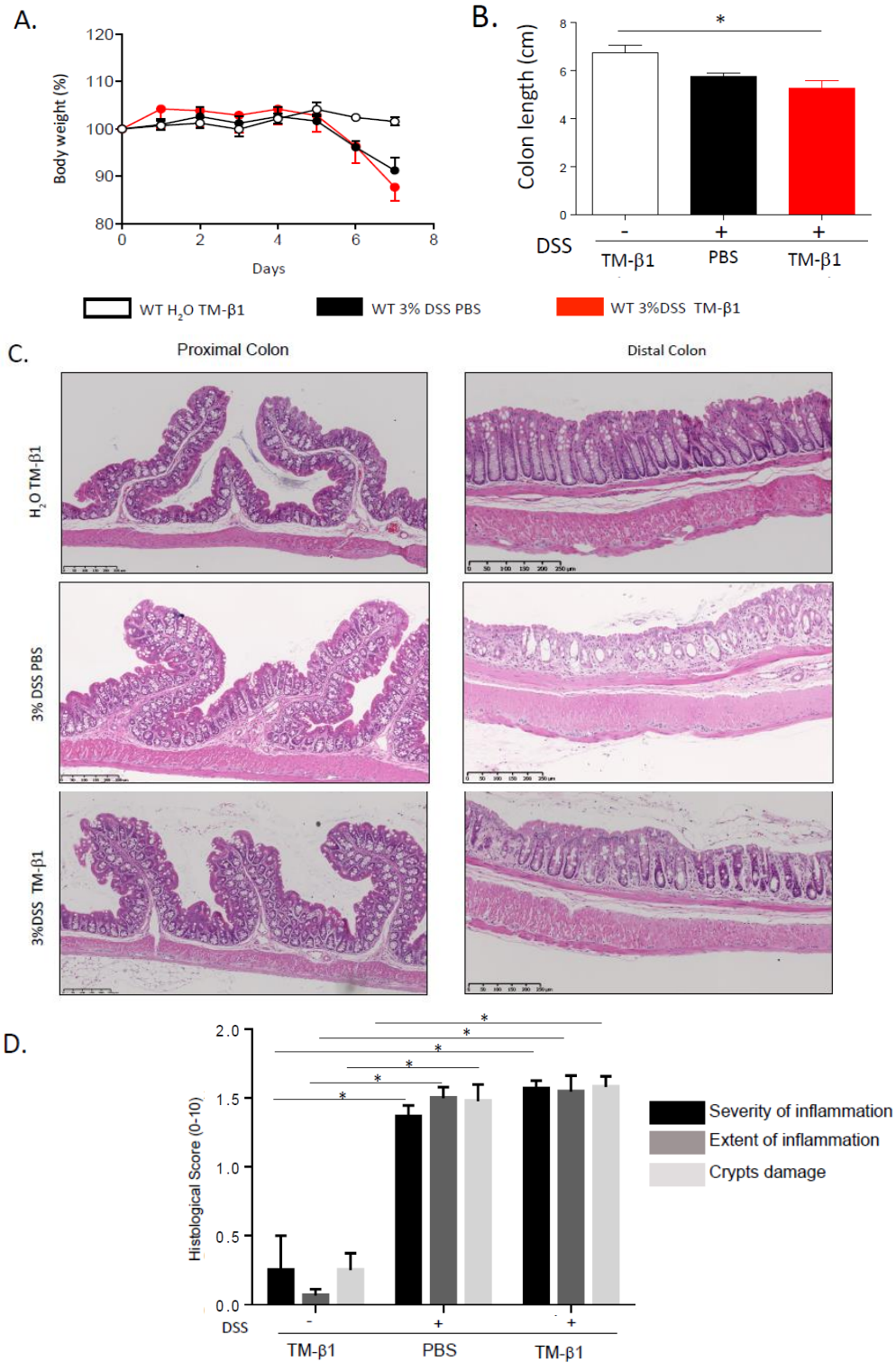

Figure 9. Anti-IL-15Rβ TM-β1 antibody does not influence DSS-induced colitis in WT mice. (A) Body weight was measured everyday of DSS treatment in WT mice treated with water or DSS, with or without TM-β1. **(B)** Colon length from PBS or antibody injected DSS treated mice. **(C)** Representative H&E sections of distal colon. Representative images from at least 4 mice for each group are shown. Magnification 10X (Scale bar represents 250 µm). **(D)** Histological score of the colon (WT TM-β1 H_2O n=4, WT PBS 3% DSS n=6, WT TM-β1 3% DSS n=6). Error bars represent the standard error of the average. Statistical significance using one-way ANOVA test is shown by * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.

3.3. Effect of inhibiting IL-15 signaling on DSS-induced colitis

Biologics that target proteins in inflammatory pathways are under various stages of development as treatment options for IBD. Targeting the IL-15 pathway might have therapeutic possibilities in both coeliac disease and IBD (Baslund *et al.*, 2005). Anti-IL-15Rβ TM-β1 can bind to the β chain of the receptor shared by cytokines IL-2 and IL-15 (Figure 3). TM-β1 blocks IL-15 signaling without interfering with IL-2 signaling (François *et al*., 1996; Nakamura *et al.*, 1993; Tanaka *et al*., 1993). Previous studies have shown that inhibiting IL-15 signaling using this antibody was beneficial in coeliac disease (Yokoyama *et al.*, 2009). Treatment with TM-β1 mAb during the initiation of the disease process, but not later, prevented the development of autoimmune diabetes in NOD mice (Bobbala *et al*., 2012). The contribution of IL-15 to the development of IBD is still not clear. It is possible that the complete knockdown of *Il15* (as described in the above experiments) could have induced compensatory mechanisms in mice rendering them equally susceptible to DSS-induced damage. Therefore, we examined the effect of blocking IL-15 signalization using TM-β1 in order to determine if neutralizing IL-15 or blocking IL-15 signaling prevents disease initiation, diminish intestinal damage, or arrest the ongoing disease, and whether these outcomes are accompanied by reduction in the disease activity index.

3.3.1. WT mice treated with TM-β1 display similar weight loss or changes in colon length following DSS treatment

Mice were pre-treated with or without TM-β1 antibody before DSS treatment. Body weight and colon length were monitored as described in the previous sections. Loss of body weight was comparable between the groups treated with DSS, irrespective of whether they received the antibody or not (Fig. 9A). Similarly, inhibition of IL-15 signaling using TM-β1 mAb did not reduce a lot the damage to the colon, as indicated by similar changes in colon length (Fig. 9B). These observations suggest that inhibition of IL-15 signaling does not affect the inflammation in the colon of WT mice treated with DSS.

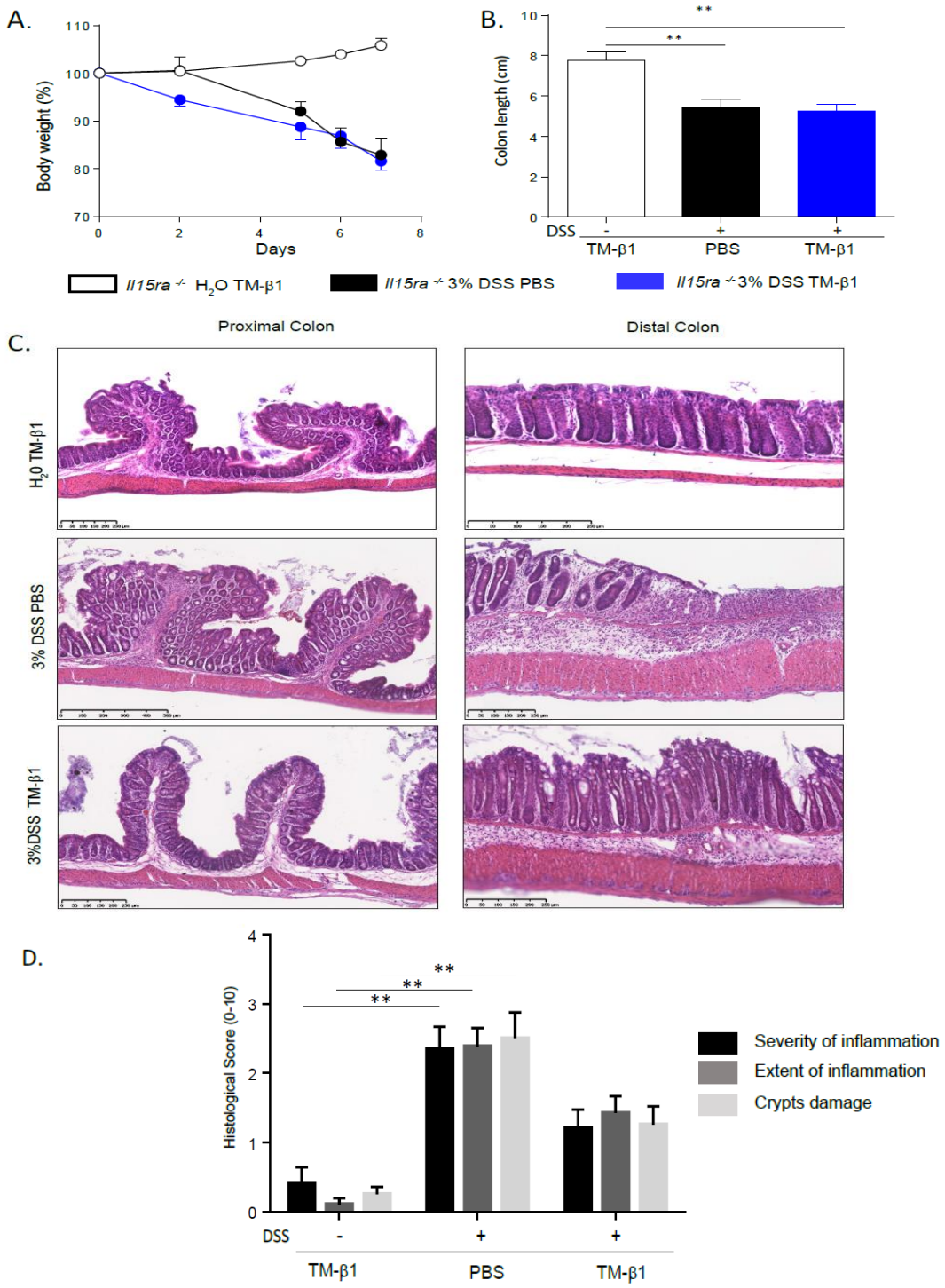

Figure 10. Anti-IL-15Rβ TM-β1 antibody protects from DSS-induced colitis in *Il15ra*$^{-/-}$ mice (A) Body weight was measured everyday of DSS treatment in *Il15ra*$^{-/-}$ mice treated with water or DSS. **(B)** Colon length was evaluated for all experimental groups. Error bars represent the standard error of the average. Statistical significance using Mann Whitney test is shown by is shown by * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.. **(C)** Sections of proximal and distal colons were stained with H&E to assess tissue inflammation in response to DSS and the antibody TM-β1. Representative images from at least 4 mice for each group are shown. Magnification 10X (Scale bar represents 250 μm). **(D)** Inflammatory histological score of the colon is significantly lower in *Il15ra*$^{-/-}$ mice treated with the TM-β1 antibody. (*Il15ra*$^{-/-}$ TM-β1 H_2O n=4, *Il15ra*$^{-/-}$ PBS 3% DSS n=6, *Il15ra*$^{-/-}$ TM-β1 3% DSS n=6). Error bars represent the standard error of the average, Statistical significance using one-way ANOVA test is shown by is shown by * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.

3.3.2. TM-β1 and PBS injected WT mice under DSS treatment are equally affected regarding the inflammatory score and epithelial architecture

We examined the epithelial architecture by H&E staining (Fig. 9C). Damage to the intestinal epithelium was comparable between mice treated or not with TM-β1 antibody. Colonic crypts showed equal damage in both groups of mice (Fig. 9D).

3.3.3. TM-β1 antibody injection induces lower macroscopic signs of intestinal inflammation in *Il15ra*$^{-/-}$ mice under DSS treatment

Previous work from the lab has shown that IL-15 can signal in the absence of IL-15Rα in certain cell types. As *Il15ra*-deficient mice have reduced intra-epithelial lymphocytes and other IL-15-dependent lymphocyte subsets, we wanted to delineate the requirement for IL-15 signaling under these conditions. Therefore, we treated *Il15ra*$^{-/-}$ mice with TM-β1 and maintained mice on DSS as described above. In this way, the binding of the cytokine to its receptor is definitively blocked. While body weight (Fig. 10A) and colon length (Fig. 10B) were not significantly affected by TM-β1 in DSS-treated WT and *Il15ra*$^{-/-}$ mutant, TM-β1 treatment decreased the damage to the intestinal tissue (Fig. 10C) and the histological score (Fig. 10D) in DSS-treated *Il15ra*-deficient mice as opposed to DSS-treated WT mice.

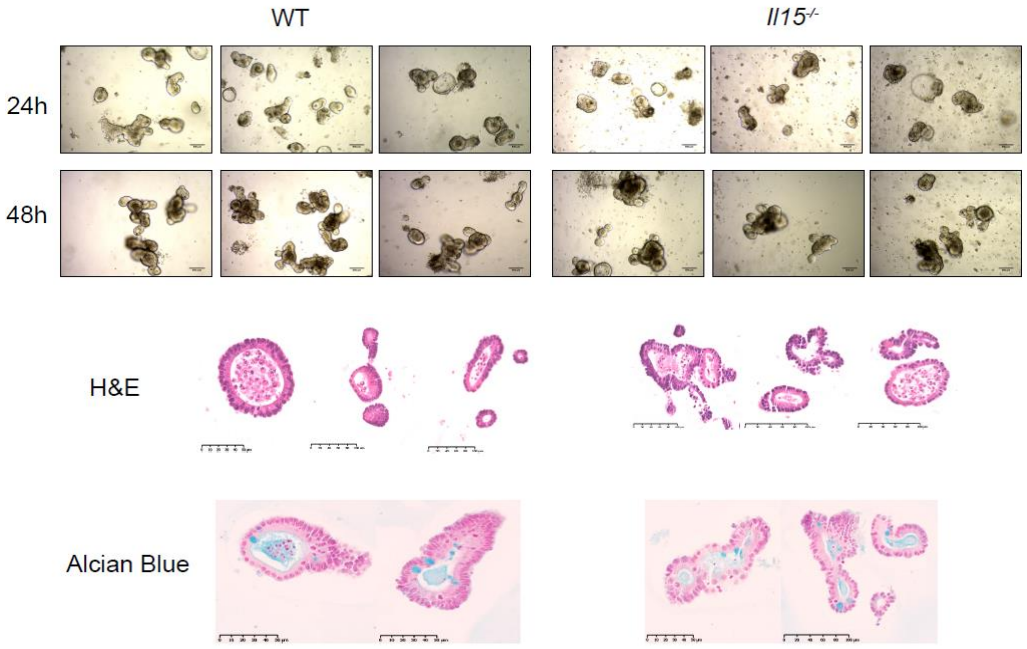

Figure 11. *Il15* deficiency does not induce morphological changes in murine enteroids. Photos were taken everyday of WT and *Il15* deficient enteroids in culture. Scale bar represents 500 µm. WT and *Il15*$^{-/-}$ enteroid sections were stained with H&E. Scale bar represents 100 µm. Acidic mucins from vesicles on goblet cells were stained with Alcian blue. Scale bar represents 100 µm.

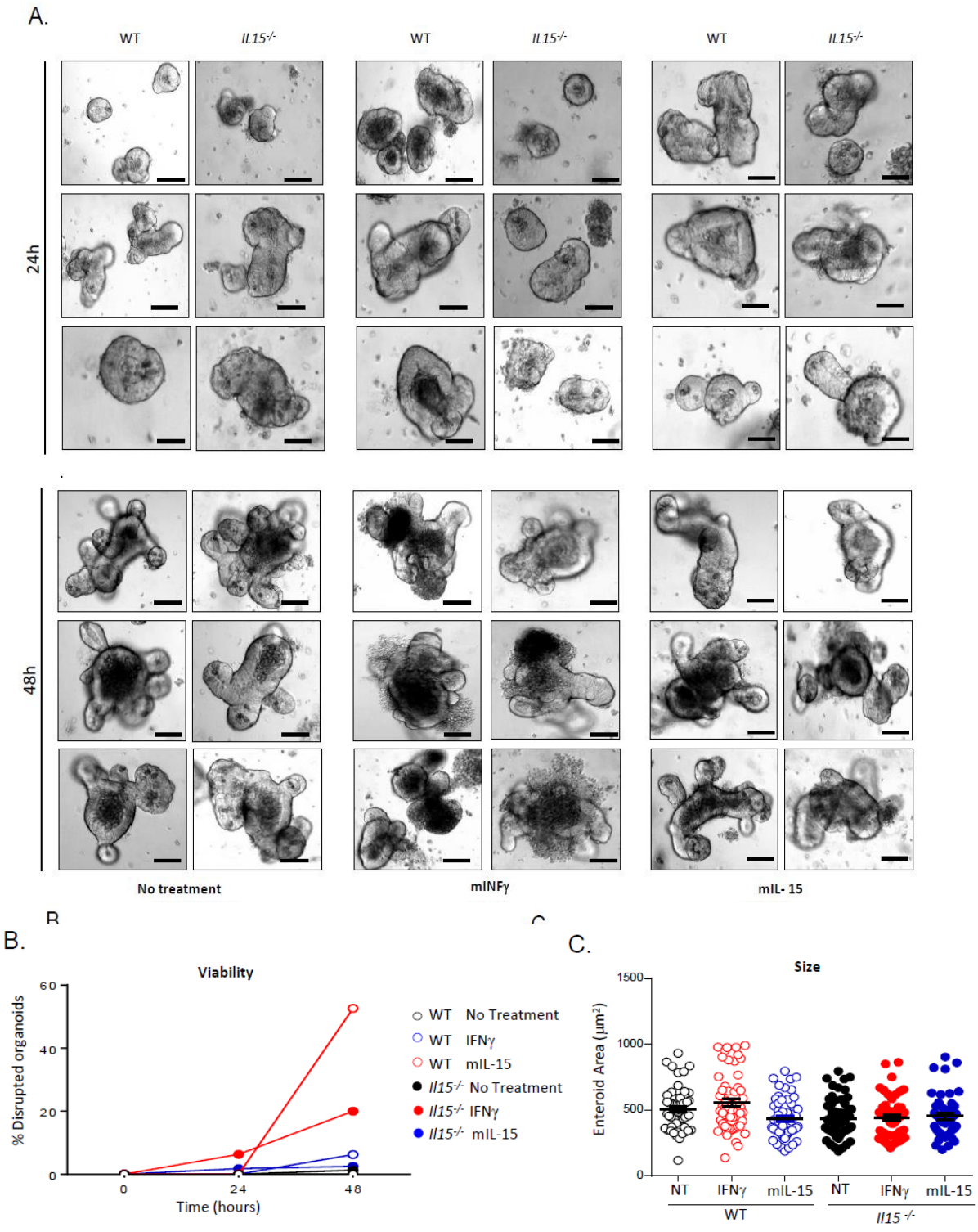

Figure 12. IFNγ treatment affects enteroid morphology and development processes. (A) Representative images from each group are shown after 24 and 48 h of treatment with IFNγ and IL-15. Scale bar represents 100 µm. **(B)** Viability represented in percent of disrupted organoids. **(C)** Organoid Size. Statistical significance using one-way ANOVA test is shown by * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.

3.4. Enteroid cell functions are modulated against an inflammatory stimulation

Enteroid 3D cell culture provide a model that resemble tissue physiology of organs *in vivo* (Sato *et al.*, 2009). Intestinal enteroids, maintained *in vitro* over long time periods, are derived from intestinal crypt cells. Enteroid cultures recreate the renewable small intestinal epithelial niche, which differentiates into different cell types, in a defined culture medium (Clevers, 2016).

3.4.1. Absence of *Il15* does not affect the development of enteroids

To determine whether autocrine IL-15 signaling played a role in crypt homeostasis, we generated enteroids from WT and *Il15*$^{-/-}$ mice and followed their morphology over a period of time by visible microscopy. We did not observe any differences in the morphology by H&E staining between enteroids obtained from WT or *Il15*$^{-/-}$ mice (Fig. 11).

3.4.2. Enteroid differentiation and development processes are significantly affected following IFNγ in WT compare with *Il15*-deficient enteroids

To identify immune cell mediators that might indirectly cause a loss of cell functions or expression of inflammatory factors after bacterial stimulation, we treated enteroids with optimal doses of proinflammatory cytokines mIFNγ and mIL-15. Enteroid survival following recombinant mIL-15 treatment in the presence or absence of *Il15* was comparable. In contrast, morphological analysis of *Il15*-deficient enteroids after treatment with recombinant IFNγ revealed an increased loss of enteroid morphology (Fig. 12A), an elevated enteroid disruption (Fig. 12B) and no differences in their relative size (Fig. 12C), as compared to WT enteroids. To evaluate if inflammatory stimuli from IFNγ or IL-15 signaling directly affect enteroid differentiation and inflammation, we determined the expression patterns of differentiation and inflammation markers by qPCR in WT and *Il15*$^{-/-}$ enteroids treated with mIFNγ or mIL-15. Non-stimulated *Il15*-deficient enteroids displayed reduced expression of *Sis*, an enterocyte marker, and *Tnfa*. Exogenous mIL-15 increased the expression of the *Lgr5* gene and reduced the

expression of *Tnfa* in *Il15*$^{-/-}$ enteroids. Interestingly, we observed that IFNγ significantly increased *Muc2* transcription in *Il15*$^{-/-}$ enteroids compared with WT enteroids, whereas the expression of *Chgb, Sis*, *Reg3b* and *Lgr5* was not significantly different after the IFNγ stimulation. Additionally, to further delineate whether impaired expression of *Il15* is needed for the induction of pro-inflammatory cytokines and chemokines, we evaluated the expression of *Tnfa* and *Cxcl10* mRNA levels after 48 h. We observed that following IFNγ treatment, mRNA expression of *Tnfa* and *Cxcl10* was lower in *Il15*$^{-/-}$ enteroids, compared with WT enteroids. This finding suggests a dependence on endogenous *Il15* for the expression of some cytokines and chemokines. We observed that IFNγ induced a 2-fold increase in *Il15ra* transcription in *Il15*$^{-/-}$ enteroids compared with WT enteroids. (Fig. 13).

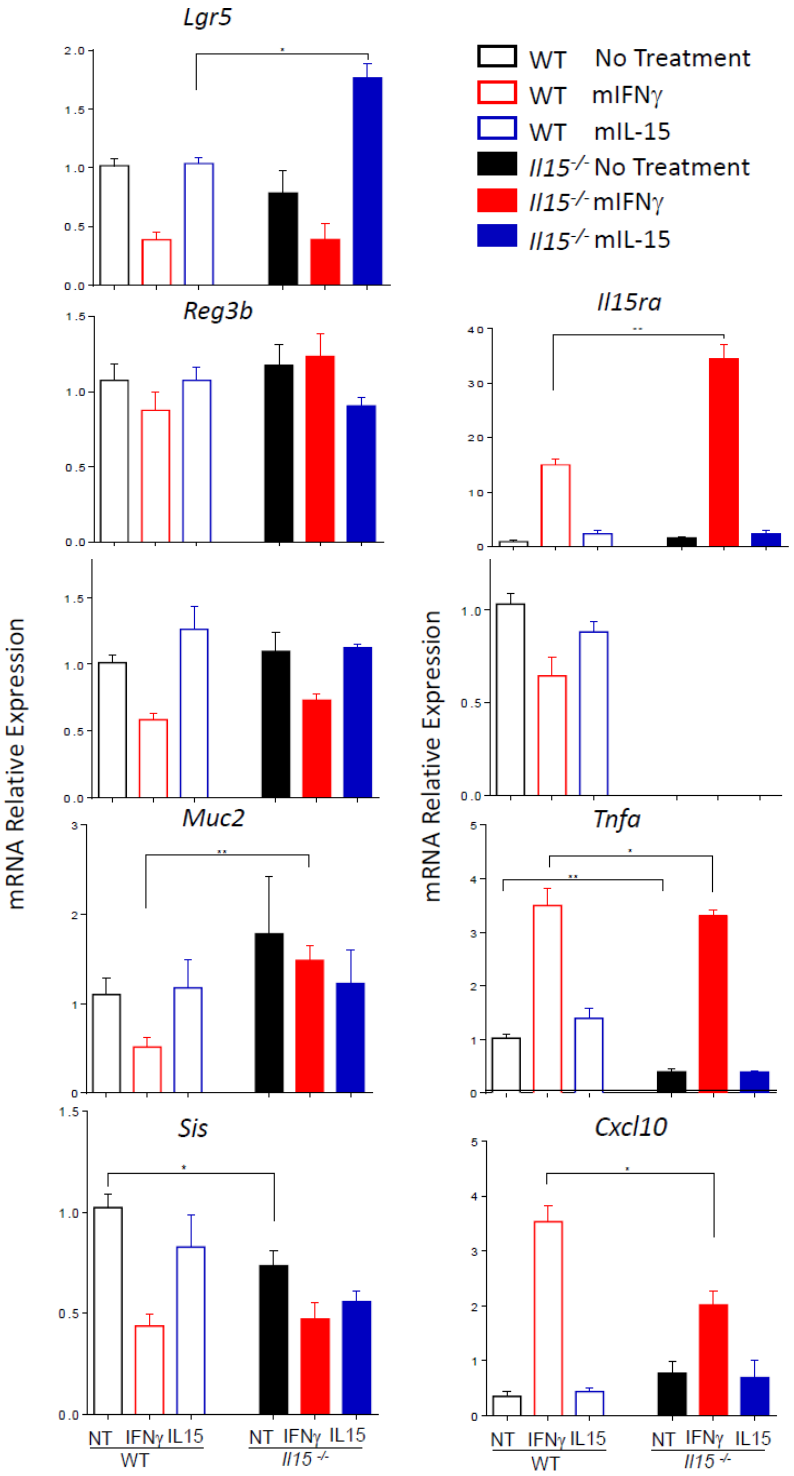

Figure 13. Expression of enteroid differentiation markers and cytokines in enteroids treated with IFNγ or IL-15. The expression of the indicated genes was studied in cultures of enteroids stimulated with IFNγ and IL-15 or non-stimulated as control for 48 hours. The data is expressed as relative expression when compared to housekeeping genes. N=2. **NT**: non-treated. Statistical significance using Mann Whitney test is shown by * $p \leq 0.05$, ** $p \leq 0.01$.

3.5. Summary of results

In this study we describe a possible role for IL-15 in IBD, particularly IL-15 expressed in IEC. We found that loss of IL-15 in the intestinal epithelium does not affect immune homeostasis in mice maintained in SPF conditions. Furthermore, we showed that *Il15*$^{-/-}$ deficient mice are equally susceptible as WT mice to develop colitis following DSS treatment. Furthermore, treatment with the anti-IL-15Rβ TM-β1, did not prevent the damage caused to the intestinal epithelium following DSS treatment in WT mice. However, in *Il15ra*-deficient mice, treatment with TM-β1 reduced the damage to the intestinal tissue.

To delineate the specific role of autocrine IL-15 in the framework of intestinal epithelial cells, we evaluated the induction of chemokines and cytokines in enteroids from small intestine. We found that HIEC can express inflammatory markers following stimulation with TLR ligands, TNFα and IFNγ. In enteroids deficiency of IL-15 can diminish the damage caused by IFNγ, and reduce the expression of chemokines and proinflammatory cytokines. Overall, our results contribute to a better understanding of the role for IL-15 in a colitis-associated inflammation.

4. DISCUSSION

The pathogenesis of Ulcerative Colitis (UC) and Crohn's disease (CD), the two principal forms of inflammatory bowel diseases (IBD), are the subject of intense research as they represent a major healthcare burden, with highest prevalence in North America and Europe and rising incidence in Asia (Ng *et al.*, 2017). IBD are characterized by a chronic relapsing inflammation, resulting from an aberrant immune response to intestinal flora in genetically susceptible individuals. The immune system is the major effector of the intestinal tissue damage, through the action of multiple cell types as well as soluble mediators, such as cytokines and chemokines.

Cytokines, key mediators of the immune system interactions in the intestinal epithelium, contribute to the disruption of the normal state of controlled inflammation (Jump and Levine, 2004). During the last years, studies on *in vivo* mouse models and human intestinal tissues have demonstrated that immune regulation, host defense pathways, epithelial barrier function, and tissue repair are important pillars of intestinal homeostasis, by controlling the host-microbe dialogue. Failure of these cytokine network pathways and their regulation can lead to IBD (Maloy and Powrie, 2010). In this work, we evaluated the role of IL-15, an inflammatory cytokine, in the physiology and pathophysiology of the intestinal mucosa.

The intestinal epithelium forms a physical and chemical barrier between the contents of the intestinal lumen and underlying cells, in addition to regulating the immune response (Peterson and Artis, 2014). It is therefore not surprising that multiple genetic defects in IEC have been reported in individuals with IBD (Khor *et al.*, 2010). To understand the cellular and molecular mechanisms by which loss of expression of IL-15 can affect the intestinal epithelium homeostasis, we analyzed cohorts of 3 to 4-month-old mice. *Il15* knockout mice did not suffer from any apparent intestinal inflammation. These results suggest that *Il15* deficiency does not induce by itself inflammation or the loss of intestinal barrier integrity. Despite the fact that mice with a genetically disrupted *Il15* gene display marked reductions in the numbers of thymic and peripheral NK T cells, memory phenotype $CD8^+$ T cells, and distinct subpopulations of intestinal IEL (Lodolce *et al.*,

1998), we found that *Il15*-deficient mice remain healthy as reported elsewhere (Kennedy *et al.*, 2000) .

Over the years, multiple studies have shown how IL-15 is implicated in several inflammatory conditions. Circulating IL-15 levels are elevated in UC and CD patients, implying a pathogenic role for IL-15 in intestinal pathologies (Kirman and Nielsen, 1996; Liu *et al.*, 2000). However, just one study has shown that absence of IL-15 protects against colitis in mice (Yoshihara *et al.*, 2006). In this study, they evaluated DSS-induced colitis in both acute and chronic phases. In acute colitis, *Il15*-deficient mice displayed lower lethality, weight loss, and reduced tissue damage as seen by histological scores. *IL15* knockout mice had reduced lamina propria $CD8^+$ T cells and NK cells, and lower levels of lamina propria proinflammatory cytokines. Additionally, in a chronic colitis model (over 30 days), similar results were found. In order to further delineate the role of IL-15 in intestinal pathology, we induced colitis with DSS in *Il15*-deficient and control mice. In contrast to this previous report (Yoshihara *et al.*, 2006), we did not observe obvious differences in the severity of the damage to intestinal tissues between WT and *Il15*-deficient mice. Both WT and *Il15*-deficient mice developed high-grade hyperplasia and dysplasia in the colon that are frequent events in colitis.

There could be various reasons to explain the observed differences. One of the major differences could be the prevalence of *Helicobacter* bacteria. Most of the animal colonies around the world harbor *Helicobacter*, *Novovirus* and other innocuous pathogens (Besselsen *et al.*, 2008). However, all the mice in our colony are free from *Helicobacter* and *Novovirus* since 2005. We speculate that these changes in gut microbiota may explain these contradictory results. Indeed, various microbiota composition in mice from different laboratories is considered to play an important role in the lack of reproducibility of data (Franklin and Ericsson, 2017). Lately, several studies have questioned whether the bacterial gut microbiota of contemporary laboratory rodents is insufficient in richness and species makeup to optimally model human conditions. It has been proposed to use gut microbiota from wild rodents or from the pet trade (Beura *et al.*, 2016; Reese *et al.*, 2016; Weldon *et al.*, 2015). Other factors that could contribute to the variance among different studies are the experimental protocol, degree of sulfate content and molecular

weight of DSS (Axelsson, 1998), and potentially other undefined environmental factors. Likewise, one should also consider the preparation of tissues for histological examination. Longitudinal sections, such as those obtained by intestinal loops (Moolenbeek and Ruitenberg, 1981), may better reflect the actual damage than cross sections because of the patchy nature of DSS-induced lesions (Cooper *et al.*, 1993).

In contrast to the above studies and our own findings, Obermeier' group has shown that neutralization of IL-15 with soluble murine Fc-IL-15Rα in chronic DSS-induced colitis resulted in disease aggravation (Obermeier *et al.*, 2006). Although the reasons for these contradictory conclusions are unclear, it is notable that Obermeier *et al*. observed an increase in intestinal epithelial damage and secretion of IL-6, TNF and IFNγ by mesenteric lymph node cells, using soluble murine IL-15Rα to block IL-15 signalization. As both mice strains are in C57BL/6 background, a possible explanation for these different findings could be the chronic DSS-induced colitis model used or the soluble IL-15Rα administered that can potentially increase the half-life of cytokines.

Oral administration of DSS in experimental animal models for the study human IBD has been shown to be different in the severity, clinical course and anatomical location of pathological changes of intestinal inflammation between different animal species. Among the laboratory animals tested, guinea pigs are the most susceptible species to DSS-induced colitis (Iwanaga *et al*., 1994). The right colon was more harshly affected than the left in hamster (Yamada *et al*., 1992) and guinea pig models (Iwanaga *et al*., 1994). In contrast, the right colon was more severely damaged in Wistar rats (Gaudio *et al*., 1999), whereas the opposite side was more damaged in Fischer 344 rats (Domek *et al*., 1995). The severity of lesions predominated in the distal colon in BALB/c and CBA/J mice, (Okayasu *et al.*, 1990), whereas the midcolon was most severely affected in Swiss-Webster mice (Cooper *et al*., 1993). We think that evaluation of different inbred strains of mice could provide the basis for investigations of the genes determining susceptibility or resistance to colitis.

In this work, we confirmed the previous observations that NOD mice are resistant to DSS-induced colitis. Our data clearly demonstrate equal sensitivity to DSS between WT

and *Il15*-deficient mice in a NOD background. Mähler's group has shown major differences in DSS responsiveness among nine strains using a standardized protocol, and have reported the resistance of NOD mice to DSS-induced lesions (Mähler *et al.*, 1998). The differences in microbiota in NOD mice tend to be lower as development of spontaneous type 1 diabetes in NOD mice is observed in 'clean' colonies only. Hence, most of the NOD colonies are maintained under 'specific pathogen free' conditions in most of the laboratories. Their results establish main differences among strains in DSS responsiveness, due either to genetic differences in the ability of the mucosa to resist inflammatory damage, differences in the ability to limit the inflammatory response, or both.

Anti-TNF monoclonal antibodies are the first broadly used biologics approved for treating both CD and UC. These antibodies, including infliximab, adalimumab, and certolizumab pegol, display good clinical efficacy (Berns and Hommes, 2016; Dryden, 2009). Nevertheless, 20% of patients under treatment do not respond, and over 30% eventually become refractory (Ungar and Kopylov, 2016). The human monoclonal antibody HuMax-IL15 targeting the β subunit of the IL-15 receptor has been well tolerated in a phase I/II clinical trial for rheumatoid arthritis, with substantial improvements in disease activity (Baslund *et al.*, 2005). This first clinical trial that directly target IL-15 in humans suggest that manipulation of the IL-15 pathway could have therapeutic possibilities in targeting IBD pathogenesis. In line with this assumption, we examined the effect of IL-2/15Rβ chain blockade *in vivo* in DSS-induced inflammation in WT and *Il15ra*$^{-/-}$ mice with the aim of understanding the role of IL-15 in intestinal inflammation. The absence of significant differences between TM-β1 treated and control mice maintained on DSS in WT mice suggests that IL-15 may not directly regulate the intestinal permeability that can promote the entry of the commensal microbiota. However, it is possible that the inflammatory process induced further to breach the epithelial barrier, is reduced in the absence of IL-15. The experiments carried out in this study have not addressed inflammatory status in DSS-treated IL-15 deficient mice or in DSS-treated mice that received TM-β1. However, certain indirect evidences suggest that targeting IL-15 signaling may have beneficial effects. For example, TM-β1

treatment diminished colon tissue destruction in *Il15ra*-deficient mice but not in WT mice maintained on DSS (Figures 9 and 10).

How to explain the differences observed between TM-β1 treated WT and *Il15ra*-deficient mice? We have shown previously that treatment with TM-β1 antibodies for 3 weeks (twice a week) prevented the development of spontaneous diabetes in NOD mice (Bobbala *et al.*, 2012). In this study, it is hypothesized that inhibition of IL-15 signaling in APC contribute to inhibit immune processes associated with the early stages of autoimmune diabetes. Additional studies in this autoimmune diabetes model suggested that in cells other than lymphocytes, IL-15 signaling does not require IL-15Ra (Bobbala *et al.*, 2017). Therefore, it is possible that the single dose of TM-β1 treatment used in this study might not have been sufficient in WT mice to inhibit the expansion and activation of lymphocyte subsets. It is well known that IL-15 signaling is required for the expansion and maintenance of IL-15-dependent lymphocytes and not necessarily for their activation. Mortier *et al*. evaluated the physiological roles of IL-15Rα-mediated trans-presentation to support NK, IEL, and $CD8^+$ T cell functions, in four lines of mice lacking IL-15Rα in the mentioned cell types. They found that NK cell survival and homeostatic proliferation mediated by IL-15Rα-IL-15 are regulated differently *in vivo*, depending on the cellular source of trans-presented IL-15 (Mortier *et al.*, 2009). Recent studies have also shown that NK cells and $CD8^+$ T cells are differently affected, while Tregs are mildly affected by inhibiting IL-2/IL-15Rβ chain (Yuan *et al.*, 2018). As IL-15-dependent lymphocytes are already absent in *Il15ra* KO mice, inhibition of IL-15 signaling might have directly inhibited the activation of innate immune cells that are not lymphocytes. In future experiments, it might be important to treat WT mice with TM-β1 for 2 weeks prior to the start of the DSS treatment.

Antibodies against pro-inflammatory cytokines, such as TNFα, IFNγ, IL-12/IL-23p40, IL-6R, IL-11, IL-13, IL-17A, as well as antibodies to integrin molecules and recombinant IFN-β and IL-10, are at various stages of clinical trials (Neurath, 2014). Anti-inflammatory cytokines, such as IL-10, have shown no positive results, as it may be more important to target pro-inflammatory processes than promote anti-inflammatory

networks. In line with these observations, our results, though preliminary, suggest that blockade of IL-15 signaling cells may have beneficial effects (Figure 10). Given the complexity of IL-15 signaling, antibodies targeting IL-15 did not show much promise in clinics (Bhaslund, 2005).

DSS-induced colitis increases the infiltration of many types of immune cells in the intestinal mucosa of WT and *Il15*-deficient mice, resulting in intestinal mucosal erosion (Sambasivarao, 2013). We have shown that inhibition of IL-15Rβ chain of the receptor partially protects the *Il15ra*-deficient mice animals treated with DSS from tissue damage (Figure 10). In *Il15*-deficient mice, it is possible that compensatory inflammatory mechanisms are in place to maintain homeostatic inflammatory processes required to protect the gut mucosa. Therefore, despite the absence of IL-15, mice are not protected from DSS-induced damage to the colon. Moreover, it has been shown that IL-15 impacts negatively butyrate-producing bacterial populations in the absence of pathology: these events precede intestinal inflammatory diseases (Meisel *et al.*, 2017).

IFNγ, associated with the T_H1 subset, is a cytokine with pleiotropic effects. Although IFNγ expression is induced in both forms of IBD, it is usually increased in the mucosa of patients with CD. IFNγ upregulates the expression of MHC class I and class II expression on macrophages and dendritic cells, increases the secretion of chemokines and activates macrophages, lymphocytes and endothelial cells (Ghosh, 2005). The second objective of this work was to study the functional consequences of proinflammatory cytokines such as IFNγ or IL-15 on IEC using enteroids. The normal crypt-villus architecture is recapitulated in organoids, which are composed of highly polarized and differentiated epithelial cells with their apical brush border facing the lumen, as well as intestinal stem cells (Date and Sato, 2015). We used this cellular model to address the role of inflammatory cytokines on IEC differentiation, and whether the absence of *Il15* could influence IEC growth, differentiation or response to cytokines. In the context of chronic or acute intestinal inflammation, the epithelium plays an important role in the overall damage by intensifying the inflammatory response induced by microbial factors. We observed that enteroid cultures from WT mice were significantly more sensitive to

proinflammatory cytokines when compared with *Il15*-deficient enteroids (Figure 12). After injury to the epithelial barrier, IEL cell populations are activated, and become a source of IFNγ (Eriguchi *et al.*, 2018). Thus, IFNγ is a potent cytokine that can cause damage to the epithelium. We observed that exogenous IFNγ increased the expression of some inflammatory mediators in enteroids. Indeed, we observed that IFNγ increases the expression of *Il15ra*, *Tnfa* and *Cxcl10* in both WT and *Il15*$^{-/-}$ enteroids. Eriguchi *et al.* (2018) showed that IFNγ (2 ng/ml) damaged enteroids within 2 days, but no other cytokine disturbed enteroid integrity. Our finding that *Il15*$^{-/-}$ enteroids were more resistant to IFNγ treatment in terms of the percentage of disrupted enteroids demonstrates that the absence of IL-15 in IEC can reduce the damage and result in the attenuation of inflammatory reaction. IFNγ treatment did not preferentially affect any particular cell type in the epithelial structure as tested by quantifying mRNAs for intestinal lineage markers. The small reduction in mRNA levels of pro-inflammatory cytokine *Tnfa* and chemokine *Cxcl10*, in the absence of IL-15 after IFNγ stimulation indicates that IL-15 expressed by enterocytes may play a role in modulating the inflammatory phenotype in IEC. Exogenous IL-15 minimally affected the expression of lineage specific markers tested in both WT and *Il15*-deficient enteroids.

Organoid cultures are a useful model the study the interactions between the intestinal epithelium and subpopulations of immune cells. For example, the generation of murine organoids and IEL co-cultures (Nozaki *et al.*, 2016) have allowed the characterization of the mechanism of motility and survival of IEL, which may be altered in the mucosal tissue of IBD patients (Ohri *et al.*, 2016). Also, co-cultures of human organoid-derived monolayers with macrophages have been used to elucidate how innate immune cells and the epithelium organize the response to enteric pathogens (Noel *et al.*, 2017). Thus, co-culture models could be used to determine the response of *Il15* or *Il15ra*-deficient organoids to *Il15* or *Il15ra*-deficient immune cells, in different combinations, in order to determine the role of IL-15 homeostasis of epithelial cells.

As discussed earlier, the etiopathogenesis of IBD remains poorly understood. Several studies strongly suggest that a dysfunctional immune response plays a key role in the

pathogenesis of IBD. Consequently, downregulation of these overactivated immune responses are the best treatment options to treat IBD. The results obtained with TM-β1 treated *Il15ra*$^{-/-}$ mice and enteroid cultures propose an approach about the pathogenic role for IL-15. Therefore, additional studies must be done with the objective of proposing IL-15 as a potential target for developing new therapies against IBD.

5. CONCLUSIONS

IBD are chronic disabling inflammatory diseases with a markedly increased incidence and prevalence in recent years. Several pro-inflammatory cytokines have been implicated in the development of IBD pathologies. The mechanisms by which IL-15 activates lymphocytes has been well-characterized. However, its role in non-immune cells, including IEC is just beginning to be studied. The current study was carried out to characterize the role of IL-15 in intestinal epithelial cell homeostasis.

The results obtained in this study in our murine model of complete invalidation of *Il15* gene demonstrate that IL-15 plays a minimal role in the maintenance of intestinal homeostasis and the mucosal protection against development of spontaneous inflammation. We showed that loss of *Il15* expression did not lead to the development of colonic intestinal inflammation, typically characterized by cryptic abscesses, acute ulceration, cell loss goblets and/or neoplasia, all of which are events associated with ulcerative colitis.

Interestingly, in the DSS-induced colitis model, we found that inhibiting IL-15 signaling by TM-β1 antibody was protective in *Il15ra*-deficient mice, but not WT mice. This suggests that IL-15 could signal independently of its receptor. IL-15 Rα could be indispensable for specific immune cells maintenance in the intestine, such as macrophages and NK cells, inducing mucosal inflammation via recruiting, maintaining and possibly activating these inflammatory cell populations.

Finally, we found that exogenous IL-15 had minimal effects on enteroids, suggesting that soluble IL-15 did not affect epithelial enteroid integrity.

During this thesis we have discussed that the blockade of IL-15 might constitute a useful target to introduce new therapeutic approaches in IBD. The role of this cytokine in the development of the intestinal inflammation can be illustrated in the Figure 14, where are observed how IELs and LP lymphocytes can strongly proliferate in response to IL-15, further leading to enhanced production IFNγ. IL-15-activated LP cells induce the production of IL-12 by monocytes-macrophages. Thus, IL-12 works in concert with

IFNγ secreted by activated T cells. IL-12 acts by inducing further IFNγ secretion by T-cells, leading to a T_H1 response, enhancing the activity of NK cells and CD8+T cells and acting as a leukocyte chemokine, thus favoring the recruitment of inflammatory cells into inflamed sites, leading to intestinal mucosa inflammation. Additionally, IL-15 also regulate the activity of anti-inflammatory pathways, including those mediated by T-regulatory cells. Accordingly, IL-15 related IL-12/ IFNγ secretion, together with the enhancing expression of CD40L on T cells mediated by IL-15, further promotes monocyte and T cells activation, recruitment of leukocytes into inflame mucosa and pro-inflammatory cytokine production, which are believed to lead to chronic inflammation and tissue damage.

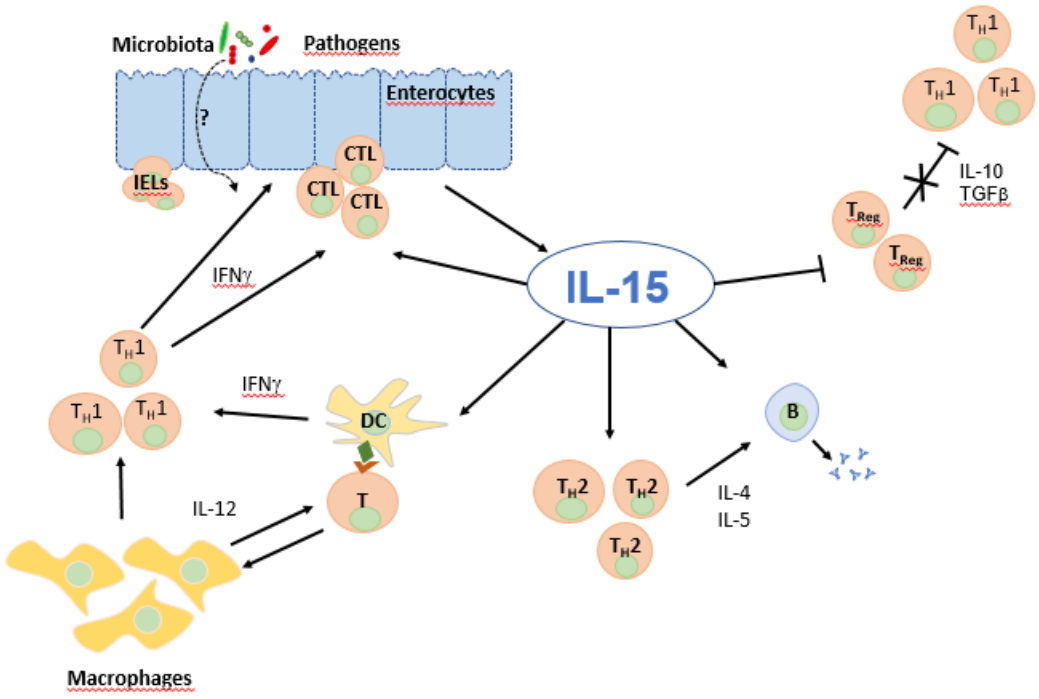

Figure 14: The central role of IL-15 in the pathogenesis of IBD. IELs: intra-epithelial lymphocytes; CTL: cytotoxic T-lymphocytes; DC: dendritic cells; T_{Reg}: T regulatory cells; T_H: T helper cells.

6. PERSPECTIVES

The results obtained in this work, however, did not allow us to conclude definitively on the implication of IL-15 in the initiation and development of IBD. It is currently controversial as to whether IL-15 protects the epithelium and/or contributes to the inflammation (Yoshihara *et al*, 2006; Obermeier *et al.*, 2006). It would be relevant to continue this project *in vivo* using chronic mouse models based on DSS-induced colitis or *Citrobacter rodentium*-induced colitis model. This will permit the analysis of the role of IL-15 during the initiation of inflammation and in the remission. It would be also interesting to evaluate the influence of IL-15 on the distinct cell lineages in the intestine of knockout mice.

Enteroid cultures could be used to focus on the role of different inflammatory cytokines on epithelial cells. Using live cell imaging, for example, we could evaluate the degranulation coupled to luminal extrusion or death of Paneth cells, for example, in response to different cytokines, including IL-15. In addition, it could be interesting to generate colonic organoids, and to compare between these organoids and enteroids, in order to understand the interactions between epithelial cells, inflammation and the gut microbiome.

7. ACKNOWLEDGEMENTS

I would first like to thank my supervisors Dr. Sheela Ramanathan and Dr. Claude Asselin, for their continuous support during my M. Sc. study and research, for their patience, motivation, enthusiasm, and immense knowledge. Their guidance helped me during research and during writing of this thesis.

Besides my supervisors, I would like to thank my "comité d'encadrement" and my evaluators, Dr. Caroline Saucier and Dr. Florian Betzinger, for taking the time to evaluate my thesis. I would like to thank to Dr. Ilangumaran as well, for the support to my project.

The door to Dr. Alfredo Menendez office was always open whenever I ran into a trouble or had a question about my research or writing. He consistently encouraged me to do better experiments each time and to critically think about the results. A very special and sincere gratitude goes out to you.

With a special mention to Marian Mayhue to whom I am extremely thankful and indebted for sharing her expertise, her sincere and valuable guidance as well as encouragement extended to me. I would like to thank the Medical Research Center of the University of Sherbrooke (CRMUS) for the financial support throughout my work.

I would also like to thank my lab mates: Sakina, Khan, Vola, Rajani, Madanraj and BV, for the stimulating discussions, for the support, and for all the fun we had in the last years… It was great sharing the laboratory with all of you. Also, I thank my colleagues at the PRAC. In particular, I am grateful to Vilcy Reyes and Alexis Gonneaud for helping me in my experiments.

Finally, I must express my very profound gratitude to my family and friends for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thanks for all your encouragement!!!

8. LIST OF REFERENCES

Abdel-Salam, B. K. A.-H. et Ebaid, H. (2008). *Upregulation of major histocompatibility complex class II, CD83, CD64, and CD14 on polymorphonuclear neutrophils stimulated with interleukin-15.*, *41*(6), 462-8.

Abreu, M. T., Fukata, M. et Arditi, M. (2005). TLR Signaling in the Gut in Health and Disease. *The Journal of Immunology*, *174*(8), 4453-4460.

Abreu, Maria T. (2010). Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. *Nature Reviews Immunology*, *11*(3), 215-215.

Ahmed, M. Ben, Hmida, N. B., Moes, N., Buyse, S., Abdeladhim, M., Louzir, H. et Cerf-Bensussan, N. (2009). IL-15 Renders Conventional Lymphocytes Resistant to Suppressive Functions of Regulatory T Cells through Activation of the Phosphatidylinositol 3-Kinase Pathway. *The Journal of Immunology*, *182*(11), 6763-6770.

Ananthakrishnan, A. N. (2015). Epidemiology and risk factors for IBD. *Nature Reviews Gastroenterology & Hepatology*, *12*(4), 205-217.

Anderson, C. A., Boucher, G., Lees, C. W., Franke, A., D'Amato, M., Taylor, K. D., … Rioux, J. D. (2011). Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nature Genetics*, *43*(3), 246-252.

Anderson, D. M., Kumaki, S., Ahdieh, M., Bertles, J., Tometsko, M., Loomis, A., … Cosman, D. (1995). Functional characterization of the human interleukin-15 receptor ?? chain and close linkage of IL15RA and IL2RA genes. *Journal of Biological Chemistry*, *270*(50), 29862-29869.

Anguille, S., Smits, E. L., Cools, N., Goossens, H., Berneman, Z. N. et Van Tendeloo, V. F. (2009). Short-term cultured, interleukin-15 differentiated dendritic cells have potent immunostimulatory properties. *Journal of Translational Medicine*, *7*(1), 109.

Annunziato, F., Cosmi, L., Santarlasci, V., Maggi, L., Liotta, F., Mazzinghi, B., … Romagnani, S. (2007). *No Title*, *204*(8).

Armitage, R. J., Macduff, B. M., Eisenman, J., Paxton, R. et Grabstein, K. H. (1995). IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. *Journal of immunology (Baltimore, Md. : 1950)*, *154*(2), 483-90.

Asseman, C., Mauze, S., Leach, M. W., Coffman, R. L. et Powrie, F. (1999). An Essential Role for Interleukin 10 in the Function of Regulatory T Cells That Inhibit Intestinal Inflammation. *The Journal of Experimental Medicine*, *190*(7), 995-1004.

Atreya, R., Mudter, J., Finotto, S., Müllberg, J., Jostock, T., Wirtz, S., … Neurath, M. F. (2000). Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: Evidence in Crohn disease and experimental colitis in vivo. *Nature Medicine*, *6*(5), 583-588.

Atreya, Raja, Zimmer, M., Bartsch, B., Waldner, M. J., Atreya, I., Neumann, H., … Neurath, M. F. (2011). Antibodies Against Tumor Necrosis Factor (TNF) Induce T-Cell Apoptosis in Patients With Inflammatory Bowel Diseases via TNF Receptor 2 and Intestinal CD14+ Macrophages. *Gastroenterology*, *141*(6), 2026-2038.

Badolato, R., Ponzi, A. N., Millesimo, M., Notarangelo, L. D. et Musso, T. (1997). Interleukin-15 (IL-15) induces IL-8 and monocyte chemotactic protein 1 production in human monocytes. *Blood*, *90*(7), 2804-9.

Barker, N. (2013). Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. *Nature Reviews Molecular Cell Biology*, *15*(1), 19-33.

Barrett, J. C., Hansoul, S., Nicolae, D. L., Cho, J. H., Duerr, R. H., Rioux, J. D., … Daly, M. J. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nature Genetics*, *40*(8), 955-962.

Baslund, B., Tvede, N., Danneskiold-Samsoe, B., Larsson, P., Panayi, G., Petersen, J., … McInnes, I. B. (2005). Targeting interleukin-15 in patients with rheumatoid arthritis: A proof-of-concept study. *Arthritis & Rheumatism*, *52*(9), 2686-2692.

Baumgart, D. C. et Sandborn, W. J. (2012). Crohn's disease. *The Lancet*, *380*(9853), 1590-1605.

Beadling, C., Guschin, D., Witthuhn, B. A., Ziemiecki, A., Ihle, J. N., Kerr, I. M. et Cantrell, D. A. (1994). Activation of JAK kinases and STAT proteins by interleukin-2 and interferon alpha, but not the T cell antigen receptor, in human T lymphocytes. *The EMBO journal*, *13*(23), 5605-15.

Beaulieu, J. F. et Quaroni, A. (1991). Clonal analysis of sucrase-isomaltase expression in the human colon adenocarcinoma Caco-2 cells. *The Biochemical journal*, *280 (Pt 3)*(3), 599-608.

Becker, C., Fantini, M. C., Schramm, C., Lehr, H. A., Wirtz, S., Nikolaev, A., … Neurath, M. F. (2004). TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity*, *21*(4), 491-501.

Becker, T. C., Wherry, E. J., Boone, D., Murali-Krishna, K., Antia, R., Ma, A. et Ahmed, R. (2002). Interleukin 15 Is Required for Proliferative Renewal of Virus-specific Memory CD8 T Cells. *The Journal of Experimental Medicine*, *195*(12), 1541-1548.

Berns, M. et Hommes, D. W. (2016). Anti-TNF-α therapies for the treatment of Crohn's disease: the past, present and future. *Expert Opinion on Investigational Drugs*, *25*(2), 129-143.

Besselsen, D. G., Franklin, C. L., Livingston, R. S. et Riley, L. K. (2008). Lurking in the shadows: emerging rodent infectious diseases. *ILAR journal*, *49*(3), 277-90.

Beura, L. K., Hamilton, S. E., Bi, K., Schenkel, J. M., Odumade, O. A., Casey, K. A., … Masopust, D. (2016). Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature*, *532*(7600), 512-516.

Biswas, A., Liu, Y.-J., Hao, L., Mizoguchi, A., Salzman, N. H., Bevins, C. L. et Kobayashi, K. S. (2010). Induction and rescue of Nod2-dependent Th1-driven granulomatous inflammation of the ileum. *Proceedings of the National Academy of Sciences*, *107*(33), 14739-14744.

Bjerknes, M. et Cheng, H. (2005). Gastrointestinal Stem Cells. II. Intestinal stem cells. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *289*(3), G381-G387.

Blaut, M. et Clavel, T. (2007). Metabolic Diversity of the Intestinal Microbiota: Implications for Health and Disease. *The Journal of Nutrition*, *137*(3), 751S-755S.

Bloemendaal, A. L. A., Buchs, N. C., George, B. D. et Guy, R. J. (2016). Intestinal stem cells and intestinal homeostasis in health and in inflammation: A review. *Surgery*, *159*(5), 1237-1248.

Bobbala, D, Chen, X., Leblanc, C., Mayhue, M. et Stankova, J. (2012). *Interleukin-15 plays an essential role in the pathogenesis of autoimmune diabetes in the NOD mouse*, 3010-3020.

Bobbala, Diwakar, Mayhue, M., Menendez, A., Ilangumaran, S. et Ramanathan, S. (2017). Trans-presentation of interleukin-15 by interleukin-15 receptor alpha is dispensable for the pathogenesis of autoimmune type 1 diabetes. *Cellular & molecular immunology*, *14*(7), 590-596.

Bouchaud, G., Mortier, E., Flamant, M., Barbieux, I., Plet, A., Galmiche, J., … Bourreille, A. (2010). Interleukin-15 and Its Soluble Receptor Mediate the Response to Infliximab in Patients With Crohn's Disease. *Gastroenterology*, *138*(7), 2378-2387.

Brande, J. M. H. Van den, Koehler, T. C., Zelinkova, Z., Bennink, R. J., Velde, A. A. te, Cate, F. J. W. ten, … Hommes, D. W. (2007). Prediction of antitumour necrosis factor clinical efficacy by real-time visualisation of apoptosis in patients with Crohn's disease. *Gut*, *56*(4), 509.

Breese, E., Braegger, C. P., Corrigan, C. J., Walker-Smith, J. A. et MacDonald, T. T. (1993). Interleukin-2- and interferon-gamma-secreting T cells in normal and diseased human intestinal mucosa. *Immunology*, *78*(1), 127-31.

Broquet, A. H., Hirata, Y., McAllister, C. S. et Kagnoff, M. F. (2011). RIG-I/MDA5/MAVS are required to signal a protective IFN response in rotavirus-infected intestinal epithelium. *Journal of immunology (Baltimore, Md. : 1950)*, *186*(3), 1618-26.

Budagian, V., Bulanova, E., Orinska, Z., Pohl, T., Borden, E. C., Silverman, R. et Bulfone-Paus, S. (2004). Reverse Signaling through Membrane-bound Interleukin-15. *Journal of Biological Chemistry*, *279*(40), 42192-42201.

Budagian, Vadim, Bulanova, E., Orinska, Z., Ludwig, A., Rose-John, S., Saftig, P., … Bulfone-Paus, S. (2004). Natural Soluble Interleukin-15Rα Is Generated by Cleavage That Involves the Tumor Necrosis Factor-α-converting Enzyme (TACE/ADAM17). *Journal of Biological Chemistry*, *279*(39), 40368-40375.

Budagian, Vadim, Bulanova, E., Paus, R. et Bulfone-Paus, S. (2006). IL-15/IL-15 receptor biology: A guided tour through an expanding universe. *Cytokine and Growth Factor Reviews*, *17*(4), 259-280.

Buffa, R., Capella, C., Fontana, P., Usellini, L. et Solcia, E. (1978). Types of endocrine cells in the human colon and rectum. *Cell and tissue research*, *192*(2), 227-40.

Bulfone-Paus, S., Bulanova, E., Budagian, V. et Paus, R. (2006). The interleukin-15/interleukin-15 receptor system as a model for juxtacrine and reverse signaling. *BioEssays*, *28*(4), 362-377.

Buonocore, S., Ahern, P. P., Uhlig, H. H., Ivanov, I. I., Littman, D. R., Maloy, K. J. et Powrie, F. (2010). Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature*, *464*(7293), 1371-1375.

Cario, E., Gerken, G. et Podolsky, D. K. (2007). Toll-Like Receptor 2 Controls Mucosal Inflammation by Regulating Epithelial Barrier Function. *Gastroenterology*, *132*(4), 1359-1374.

Casetti, R., Agrati, C., Wallace, M., Sacchi, A., Martini, F., Martino, A., … Malkovsky, M. (2009). Cutting edge: TGF-beta1 and IL-15 Induce FOXP3+ gammadelta regulatory T cells in the presence of antigen stimulation. *Journal of immunology (Baltimore, Md. : 1950)*, *183*(6), 3574-7.

Chami, B., Yeung, A. W. S., van Vreden, C., King, N. J. C. et Bao, S. (2014). The Role of CXCR3

in DSS-Induced Colitis. *PLoS ONE*, *9*(7), e101622. doi: 10.1371/journal.pone.0101622

Chassaing, B. et Darfeuille–Michaud, A. (2011). The Commensal Microbiota and Enteropathogens in the Pathogenesis of Inflammatory Bowel Diseases. *Gastroenterology*, *140*(6), 1720-1728.e3.

Chen, G. Y. et Núñez, G. (2011). Inflammasomes in intestinal inflammation and cancer. *Gastroenterology*, *141*(6), 1986-99.

Cheroutre, H., Lambolez, F. et Mucida, D. (2011). The light and dark sides of intestinal intraepithelial lymphocytes. *Nature Reviews Immunology*, *11*(7), 445-456.

Cianci, R., Pagliari, D., Pietroni, V., Landolfi, R. et Pandolfi, F. (2010). Tissue infiltrating lymphocytes: the role of cytokines in their growth and differentiation. *Journal of biological regulators and homeostatic agents*, *24*(3), 239-49.

Clevers, H. (2016). Modeling Development and Disease with Organoids. *Cell*, *165*(7), 1586-1597.

Cobo, E. et Chadee, K. (2013). Antimicrobial Human β-Defensins in the Colon and Their Role in Infectious and Non-Infectious Diseases. *Pathogens*, *2*(1), 177-192.

Cook, D. N., Prosser, D. M., Forster, R., Zhang, J., Kuklin, N. A., Abbondanzo, S. J., … Lira, S. A. (2000). CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue. *Immunity*, *12*(5), 495-503.

Cooper, H. S., Murthy, S. N., Shah, R. S. et Sedergran, D. J. (1993). Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Laboratory investigation; a journal of technical methods and pathology*, *69*(2), 238-49.

Crosnier, C., Stamataki, D. et Lewis, J. (2006). Organizing cell renewal in the intestine: Stem cells, signals and combinatorial control. *Nature Reviews Genetics*, *7*(5), 349-359.

Crowe, P. T. et Marsh, M. N. (1994). Morphometric analysis of intestinal mucosa. VI ? Principles in enumerating intra-epithelial lymphocytes. *Virchows Archiv*, *424*(3), 301-306.

Cukrowska, B., Sowińska, A., Bierła, J. B., Czarnowska, E., Rybak, A. et Grzybowska-Chlebowczyk, U. (2017, 14 novembre). Intestinal epithelium, intraepithelial lymphocytes and the gut microbiota - Key players in the pathogenesis of celiac disease (vol. 23, p. 7505-7518). Baishideng Publishing Group Co., Limited.

Danese, S. et Fiocchi, C. (2011). Ulcerative Colitis. *New England Journal of Medicine*, *365*(18), 1713-1725.

Date, S. et Sato, T. (2015). Mini-Gut Organoids: Reconstitution of the Stem Cell Niche. *Annual Review of Cell and Developmental Biology*, *31*(1), 269-289.

Dedhia, P. H., Bertaux-Skeirik, N., Zavros, Y. et Spence, J. R. (2016). Organoid Models of Human Gastrointestinal Development and Disease. *Gastroenterology*, *150*(5), 1098-1112.

Demirci, G. et Li, X. C. (2004). IL-2 and IL-15 exhibit opposing effects on Fas mediated apoptosis. *Cellular & molecular immunology*, *1*(2), 123-8.

Di Sabatino, A., Calarota, S. A., Vidali, F., MacDonald, T. T. et Corazza, G. R. (2011). Role of IL-15 in immune-mediated and infectious diseases. *Cytokine and Growth Factor Reviews*, *22*(1), 19-33.

Di Sabatino, A., Pender, S. L. F., Jackson, C. L., Prothero, J. D., Gordon, J. N., Picariello, L., …

MacDonald, T. T. (2007). Functional Modulation of Crohn's Disease Myofibroblasts by Anti-Tumor Necrosis Factor Antibodies. *Gastroenterology*, *133*(1), 137-149.

Dieleman, L. A., Elson, C. O., Tennyson, G. S. et Beagley, K. W. (1996). Kinetics of cytokine expression during healing of acute colitis in mice. *The American journal of physiology*, *271*(1 Pt 1), G130-6.

Dieleman, L. A., Palmen, M. J., Akol, H., Bloemena, E., PENA, A. S., Meuwissen, S. G. et Van Rees, E. P. (1998). Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clinical and Experimental Immunology*, *114*(3), 385-391.

Dieleman, L. A., Ridwan, B. U., Tennyson, G. S., Beagley, K. W., Bucy, R. P. et Elson, C. O. (1994). Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology*, *107*(6), 1643-52.

Domek, M. J., Iwata, F., Blackman, E. I., Kao, J., Baker, M., Vidrich, A. et Leung, F. W. (1995). Anti-Neutrophil Serum Attenuates Dextran Sulfate Sodium-Induced Colonic Damage in the Rat. *Scandinavian Journal of Gastroenterology*, *30*(11), 1089-1094.

Dorofeyev, A. E., Vasilenko, I. V, Rassokhina, O. A. et Kondratiuk, R. B. (2013). Mucosal barrier in ulcerative colitis and Crohn's disease. *Gastroenterology research and practice*, *2013*, 431231.

Dryden, G. W. (2009). Overview of biologic therapy for Crohn's disease. *Expert Opinion on Biological Therapy*, *9*(8), 967-974.

Dubois, S, Magrangeas, F., Lehours, P., Raher, S., Bernard, J., Boisteau, O., … Jacques, Y. (1999). Natural splicing of exon 2 of human interleukin-15 receptor alpha-chain mRNA results in a shortened form with a distinct pattern of expression. *The Journal of biological chemistry*, *274*(38), 26978-84.

Dubois, Sigrid, Mariner, J., Waldmann, T. A. et Tagaya, Y. (2002). IL-15Rα Recycles and Presents IL-15 In trans to Neighboring Cells. *Immunity*, *17*(5), 537-547.

Duerr, R. H., Taylor, K. D., Brant, S. R., Rioux, J. D., Silverberg, M. S., Daly, M. J., … Cho, J. H. (2006). A Genome-Wide Association Study Identifies IL23R as an Inflammatory Bowel Disease Gene. *Science*, *314*(5804), 1461-1463.

Dye, B. R., Hill, D. R., Ferguson, M. A., Tsai, Y.-H., Nagy, M. S., Dyal, R., … Spence, J. R. (2015). In vitro generation of human pluripotent stem cell derived lung organoids. *eLife*, *4*.

Ebert, E. C. (1998). Interleukin 15 is a potent stimulant of intraepithelial lymphocytes. *Gastroenterology*, *115*(6), 1439-45.

Edelblum, K. L., Shen, L., Weber, C. R., Marchiando, A. M., Clay, B. S., Wang, Y., … Turner, J. R. (2012). Dynamic migration of γδ intraepithelial lymphocytes requires occludin. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(18), 7097-102.

Edelblum, K. L., Yan, F., Yamaoka, T. et Polk, B. D. (2006). Regulation of apoptosis during homeostasis and disease in the intestinal epithelium. *Inflammatory Bowel Diseases*, *12*(5), 413-424.

Elinav, E., Nowarski, R., Thaiss, C. A., Hu, B., Jin, C. et Flavell, R. A. (2013). Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nature*

Reviews Cancer, *13*(11), 759-771.

Eriguchi, Y., Nakamura, K., Yokoi, Y., Sugimoto, R., Takahashi, S., Hashimoto, D., … Ouellette, A. J. (2018). Essential role of IFN-γ in T cell–associated intestinal inflammation. *JCI Insight*, *3*(18).

Evans, G. S. et Potten, C. S. (1988). The distribution of endocrine cells along the mouse intestine: a quantitative immunocytochemical study. *Virchows Archiv. B, Cell pathology including molecular pathology*, *56*(3), 191-9.

Fais, S., Capobianchi, M. R., Pallone, F., Marco, P. Di, Boirivant, M., Dianzani, F., … Mater Domini, P. (1991). No Title. *Gut*, *32*(4).

Fantini, M. C., Rizzo, A., Fina, D., Caruso, R., Sarra, M., Stolfi, C., … Monteleone, G. (2009). Smad7 Controls Resistance of Colitogenic T Cells to Regulatory T Cell-Mediated Suppression. *Gastroenterology*, *136*(4), 1308-1316.e3.

Fatehullah, A., Tan, S. H. et Barker, N. (2016). Organoids as an in vitro model of human development and disease. *Nature Cell Biology*, *18*(3), 246-254.

Feau, S., Facchinetti, V., Granucci, F., Citterio, S., Jarrossay, D., Seresini, S., … Ricciardi-Castagnoli, P. (2005). Dendritic cell-derived IL-2 production is regulated by IL-15 in humans and in mice. *Blood*, *105*(2), 697-702.

Fehniger, T. A. et Caligiuri, M. A. (2001). Interleukin 15: biology and relevance to human disease. *Blood*, *97*(1), 14-32.

Ferraris, R. P., Villenas, S. A. et Diamond, J. (1992). Regulation of brush-border enzyme activities and enterocyte migration rates in mouse small intestine. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *262*(6), G1047-G1059.

François, C., Dantal, J., Sorel, M., Cherel, M., Miyasaka, M., Soulillou, J. P. et Jacques, Y. (1996). Antibodies directed at mouse IL-2-R alpha and beta chains act in synergy to abolish T-cell proliferation in vitro and delayed type hypersensitivity reaction in vivo. *Transplant international : official journal of the European Society for Organ Transplantation*, *9*(1), 46-50.

Frank, D. N., St. Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N. et Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences*, *104*(34), 13780-13785.

Franklin, C. L. et Ericsson, A. C. (2017). Microbiota and reproducibility of rodent models. *Lab animal*, *46*(4), 114-122.

Fujimura, Y. et Iida, M. (2001). A new marker for cup cells in the rabbit small intestine: expression of vimentin intermediate filament protein. *Medical electron microscopy : official journal of the Clinical Electron Microscopy Society of Japan*, *34*(4), 223-9.

Fukata, M., Michelsen, K. S., Eri, R., Thomas, L. S., Hu, B., Lukasek, K., … Abreu, M. T. (2005). Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *288*(5), G1055-G1065.

Fuss, I. J., Neurath, M., Boirivant, M., Klein, J. S., de la Motte, C., Strong, S. A., … Strober, W. (1996). Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory

bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *Journal of immunology (Baltimore, Md. : 1950)*, *157*(3), 1261-70.

Gaggero, A., Azzarone, B., Andrei, C., Mishal, Z., Meazza, R., Zappia, E., … Ferrini, S. (1999). Differential intracellular trafficking, secretion and endosomal localization of two IL-15 isoforms. *European journal of immunology*, *29*(4), 1265-74.

Gajda, A. M. et Storch, J. (2015). Enterocyte fatty acid-binding proteins (FABPs): Different functions of liver and intestinal FABPs in the intestine. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *93*, 9-16.

Gaudio, E., Taddei, G., Vetuschi, A., Sferra, R., Frieri, G., Ricciardi, G. et Caprilli, R. (1999). Dextran Sulfate Sodium (DSS) Colitis in Rats (Clinical, Structural, and Ultrastructural Aspects). *Digestive Diseases and Sciences*, *44*(7), 1458-1475.

Gebhardt, T., Mueller, S. N., Heath, W. R. et Carbone, F. R. (2013). Peripheral tissue surveillance and residency by memory T cells. *Trends in immunology*, *34*(1), 27-32.

Gewirtz, A. T., Navas, T. A., Lyons, S., Godowski, P. J. et Madara, J. L. (2001). Cutting Edge: Bacterial Flagellin Activates Basolaterally Expressed TLR5 to Induce Epithelial Proinflammatory Gene Expression. *The Journal of Immunology*, *167*(4), 1882-1885.

Ghosh, S. (2005). Interfering with interferons in inflammatory bowel disease. *Gut*, *55*(8), 1071-1073.

Giri, J. G., Kumaki, S., Ahdieh, M., Friend, D. J., Loomis, A., Shanebeck, K., … Anderson, D. M. (1995). Identification and cloning of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor. *The EMBO journal*, *14*(15), 3654-63.

Grabstein, K. H., Eisenman, J., Shanebeck, K., Rauch, C., Srinivasan, S., Fung, V., … Ahdieh, M. (1994). Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science (New York, N.Y.)*, *264*(5161), 965-8.

Gracia-Sancho, J. et Salvadó, M. J. (2017). *Gastrointestinal tissue : oxidative stress and dietary antioxidants*. London : : Elsevier/Academic Press, an imprint of Elsevier,.

Groschwitz, K. R. et Hogan, S. P. (2009). Intestinal barrier function: Molecular regulation and disease pathogenesis. *Journal of Allergy and Clinical Immunology*, *124*(1), 3-20.

Gu, H., Maeda, H., Moon, J. J., Lord, J. D., Yoakim, M., Nelson, B. H. et Neel, B. G. (2000). New role for Shc in activation of the phosphatidylinositol 3-kinase/Akt pathway. *Molecular and cellular biology*, *20*(19), 7109-20.

Gunawardene, A. R., Corfe, B. M. et Staton, C. A. (2011). Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. *International Journal of Experimental Pathology*, *92*(4), 219-231.

Günther, C., Martini, E., Wittkopf, N., Amann, K., Weigmann, B., Neumann, H., … Becker, C. (2011). Caspase-8 regulates TNF-α-induced epithelial necroptosis and terminal ileitis. *Nature*, *477*(7364), 335-339.

Hatakeyama, M., Mori, H., Doi, T. et Taniguchi, T. (1989). A restricted cytoplasmic region of IL-2 receptor beta chain is essential for growth signal transduction but not for ligand binding and internalization. *Cell*, *59*(5), 837-45.

Hauri, H. P., Sterchi, E. E., Bienz, D., Fransen, J. A. et Marxer, A. (1985). Expression and

intracellular transport of microvillus membrane hydrolases in human intestinal epithelial cells. *The Journal of Cell Biology*, *101*(3), 838-851.

Heller, F., Florian, P., Bojarski, C., Richter, J., Christ, M., Hillenbrand, B., … Schulzke, J. D. (2005). Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology*, *129*(2), 550-64.

Hermiston, M. L. et Gordon, J. I. (1995). In vivo analysis of cadherin function in the mouse intestinal epithelium: essential roles in adhesion, maintenance of differentiation, and regulation of programmed cell death. *The Journal of Cell Biology*, *129*(2), 489-506.

Herrlinger, K. R., Witthoeft, T., Raedler, A., Bokemeyer, B., Krummenerl, T., Schulzke, J.-D., … Stange, E. F. (2006). Randomized, Double Blind Controlled Trial of Subcutaneous Recombinant Human Interleukin-11 Versus Prednisolone in Active Crohn's Disease. *The American Journal of Gastroenterology*, *101*(4), 793-797.

Hoontrakoon, R., Chu, H. W., Gardai, S. J., Wenzel, S. E., McDonald, P., Fadok, V. A., … Bratton, D. L. (2002). Interleukin-15 Inhibits Spontaneous Apoptosis in Human Eosinophils via Autocrine Production of Granulocyte Macrophage–Colony Stimulating Factor and Nuclear Factor- κ B Activation. *American Journal of Respiratory Cell and Molecular Biology*, *26*(4), 404-412.

Huber, S., Gagliani, N., Esplugues, E., O'Connor, W., Huber, F. J., Chaudhry, A., … Flavell, R. A. (2011). Th17 Cells Express Interleukin-10 Receptor and Are Controlled by Foxp3− and Foxp3+ Regulatory CD4+ T Cells in an Interleukin-10-Dependent Manner. *Immunity*, *34*(4), 554-565.

Hugot, J.-P., Chamaillard, M., Zouali, H., Lesage, S., Cézard, J.-P., Belaiche, J., … Thomas, G. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*, *411*(6837), 599-603.

Huntington, N. D., Legrand, N., Alves, N. L., Jaron, B., Weijer, K., Plet, A., … Di Santo, J. P. (2009). IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. *The Journal of experimental medicine*, *206*(1), 25-34.

Imamichi, H., Sereti, I. et Lane, H. C. (2008). IL-15 acts as a potent inducer of CD4+CD25hi cells expressing FOXP3. *European Journal of Immunology*, *38*(6), 1621-1630.

Inagaki-Ohara, K., Nishimura, H., Mitani, A. et Yoshikai, Y. (1997). Interleukin-15 preferentially promotes the growth of intestinal intraepithelial lymphocytes bearing γδ T cell receptor in mice. *European Journal of Immunology*, *27*(11), 2885-2891.

Ismail, A. S., Severson, K. M., Vaishnava, S., Behrendt, C. L., Yu, X., Benjamin, J. L., … Hooper, L. V. (2011). intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *Proceedings of the National Academy of Sciences*, *108*(21), 8743-8748.

Ivanov, I. I., Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., … Littman, D. R. (2009). Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell*, *139*(3), 485-498.

Iwanaga, T., Hoshi, O., Han, H. et Fujita, T. (1994). Morphological analysis of acute ulcerative colitis experimentally induced by dextran sulfate sodium in the guinea pig: some possible mechanisms of cecal ulceration. *Journal of gastroenterology*, *29*(4), 430-8.

Izcue, A., Coombes, J. L. et Powrie, F. (2009). Regulatory Lymphocytes and Intestinal

Inflammation. *Annual Review of Immunology*, *27*(1), 313-338.

Johansen, F-E et Kaetzel, C. S. (2011). Regulation of the polymeric immunoglobulin receptor and IgA transport: new advances in environmental factors that stimulate pIgR expression and its role in mucosal immunity. *Mucosal Immunology*, *4*(6), 598-602.

Johansen, Finn-Eirik, Pekna, M., Norderhaug, I. N., Haneberg, B., Hietala, M. A., Krajci, P., … Brandtzaeg, P. (1999). Absence of Epithelial Immunoglobulin a Transport, with Increased Mucosal Leakiness, in Polymeric Immunoglobulin Receptor/Secretory Component–Deficient Mice. *The Journal of Experimental Medicine*, *190*(7), 915-922.

Johansson, M. E. V, Phillipson, M., Petersson, J., Velcich, A., Holm, L. et Hansson, G. C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(39), 15064-9.

Jostins, L., Ripke, S., Weersma, R. K., Duerr, R. H., McGovern, D. P., Hui, K. Y., … Cho, J. H. (2012). Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*, *491*(7422), 119-124.

Jump, R. L. et Levine, A. D. (2004). Mechanisms of Natural Tolerance in the Intestine. *Inflammatory Bowel Diseases*, *10*(4), 462-478.

K Yoshihara , T Yajima, C Kubo, Y. Y. (2006). Role of interleukin 15 in colitis induced by dextran sulphate sodium in mice. *Gut*, *55*(3), 334-341.

Kagnoff, M. F. (2007). Mucosal Inflammation in Celiac Disease: Interleukin-15 Meets Transforming Growth Factor β-1. *Gastroenterology*, *132*(3), 1174-1176.

Kaiser, G. C. et Polk, D. B. (1997). Tumor necrosis factor alpha regulates proliferation in a mouse intestinal cell line. *Gastroenterology*, *112*(4), 1231-40.

Kapp, J. A., Kapp, L. M. et McKenna, K. C. (2004). γδ T Cells Play an Essential Role in Several Forms of Tolerance. *Immunologic Research*, *29*(1-3), 093-102.

Kaser, A., Zeissig, S. et Blumberg, R. S. (2010). Inflammatory Bowel Disease. *Annual Review of Immunology*, *28*(1), 573-621.

Kellett, G. L., Brot-Laroche, E., Mace, O. J. et Leturque, A. (2008). Sugar Absorption in the Intestine: The Role of GLUT2. *Annual Review of Nutrition*, *28*(1), 35-54.

Kennedy, M. K., Glaccum, M., Brown, S. N., Butz, E. A., Viney, J. L., Embers, M., … Peschon, J. J. (2000). Reversible Defects in Natural Killer and Memory Cd8 T Cell Lineages in Interleukin 15–Deficient Mice. *The Journal of Experimental Medicine*, *191*(5), 771-780.

Khor, B., Gardet, A. et Xavier, R. J. (2011). Genetics and pathogenesis of inflammatory bowel disease. *Nature*, *474*(7351), 307-317.

Kim, B. S., Siracusa, M. C., Saenz, S. A., Noti, M., Monticelli, L. A., Sonnenberg, G. F., … Artis, D. (2013). TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Science translational medicine*, *5*(170), 170ra16.

Kim, J. J., Shajib, M. S., Manocha, M. M. et Khan, W. I. (2012). Investigating Intestinal Inflammation in DSS-induced Model of IBD. *Journal of Visualized Experiments*, (60), 1-7.

Kirman, I. et Nielsen, O. H. (1996). Increased numbers of interleukin-15-expressing cells in active ulcerative colitis. *The American journal of gastroenterology*, *91*(9), 1789-94.

Kobayashi, K. S., Chamaillard, M., Ogura, Y., Henegariu, O., Inohara, N., Nuñez, G. et Flavell, R. A. (2005). Nod2-Dependent Regulation of Innate and Adaptive Immunity in the Intestinal Tract. *Science*, *307*(5710), 731-734.

Kole, A. et Maloy, K. J. (2014). Control of Intestinal Inflammation by Interleukin-10. Dans *Current topics in microbiology and immunology* (vol. 380, p. 19-38).

Krause, H., Jandrig, B., Wernicke, C., Bulfone-Paus, S., Pohl, T. et Diamantstein, T. (1996). Genomic structure and chromosomal localization of the human interleukin 15 gene (IL–15). *Cytokine*, *8*(9), 667-674.

Krebs, D. L. et Hilton, D. J. (2001). SOCS Proteins: Negative Regulators of Cytokine Signaling. *Stem Cells*, *19*(5), 378-387.

Krieglstein, C. F., Cerwinka, W. H., Sprague, A. G., Laroux, F. S., Grisham, M. B., Koteliansky, V. E., … de Fougerolles, A. R. (2002). Collagen-binding integrin α1β1 regulates intestinal inflammation in experimental colitis. *Journal of Clinical Investigation*, *110*(12), 1773-1782.

Kühn, R., Löhler, J., Rennick, D., Rajewsky, K. et Müller, W. (1993). Interleukin-10-deficient mice develop chronic enterocolitis. *Cell*, *75*(2), 263-74.

L.-G. Axelsson, E. L. & A.-C. B.-F. (1998). Experimental colitis induced by dextran sulphate sodium in mice : bene ® cial effects of sulphasalazine and olsalazine. *Alimentary Pharmacology and Therapeutics*, (12), 925-934.

Lala, S., Ogura, Y., Osborne, C., Hor, S. Y., Bromfield, A., Davies, S., … Keshav, S. (2003). Crohn's disease and the NOD2 gene: a role for paneth cells. *Gastroenterology*, *125*(1), 47-57.

Lancaster, M. A. et Knoblich, J. A. (2014). Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science*, *345*(6194), 1247125-1247125.

Landy, J., Ronde, E., English, N., Clark, S. K., Hart, A. L., Knight, S. C., … Al-Hassi, H. O. (2016). Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer. *World Journal of Gastroenterology*, *22*(11), 3117.

Laukoetter, M. G., Nava, P. et Nusrat, A. (2008). Role of the intestinal barrier in inflammatory bowel disease. *World Journal of Gastroenterology : WJG*, *14*(3), 401.

Lazarevic, V., Glimcher, L. H. et Lord, G. M. (2013). T-bet: a bridge between innate and adaptive immunity. *Nature Reviews Immunology*, *13*(11), 777-789.

Leonard, W J. (2001). Role of Jak kinases and STATs in cytokine signal transduction. *International journal of hematology*, *73*(3), 271-7.

Leonard, Warren J. (2001). Cytokines and immunodeficiency diseases. *Nature Reviews Immunology*, *1*(3), 200-208.

Lie Bouchard, A., Ratthé, C. et Girard, D. (2004). Interleukin-15 delays human neutrophil apoptosis by intracellular events and not via extracellular factors: role of Mcl-1 and decreased activity of caspase-3 and caspase-8. *Journal of Leukocyte Biology*, *75*(5), 893-900.

Lindén, S. K., Florin, T. H. J. et McGuckin, M. A. (2008). Mucin Dynamics in Intestinal Bacterial Infection. *PLoS ONE*, *3*(12), e3952.

Liu, Z., Geboes, K., Colpaert, S., D'Haens, G. R., Rutgeerts, P. et Ceuppens, J. L. (2000). IL-15

Is Highly Expressed in Inflammatory Bowel Disease and Regulates Local T Cell-Dependent Cytokine Production. *The Journal of Immunology*, *164*(7), 3608-3615.

Lodolce, J P, Boone, D. L., Chai, S., Swain, R. E., Dassopoulos, T., Trettin, S. et Ma, A. (1998). IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity*, *9*(5), 669-76.

Lodolce, James P., Burkett, P. R., Boone, D. L., Chien, M. et Ma, A. (2001). T Cell–Independent Interleukin 15rα Signals Are Required for Bystander Proliferation. *The Journal of Experimental Medicine*, *194*(8), 1187-1194.

Loo, Y.-M., Gale, M. et Jr. (2011). Immune signaling by RIG-I-like receptors. *Immunity*, *34*(5), 680-92.

Macpherson, A. J. et Uhr, T. (2004). Induction of Protective IgA by Intestinal Dendritic Cells Carrying Commensal Bacteria. *Science*, *303*(5664), 1662-1665.

Macpherson, Andrew J. et Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews Immunology*, *4*(6), 478-485.

Madsen, K. L., Malfair, D., Gray, D., Doyle, J. S., Jewell, L. D. et Fedorak, R. N. (1999). Interleukin-10 Gene-Deficient Mice Develop a Primary Intestinal Permeability Defect in Response to Enteric Microflora. *Inflammatory Bowel Diseases*, *5*(4), 262-270.

Maeda, S., Hsu, L.-C., Liu, H., Bankston, L. A., Iimura, M., Kagnoff, M. F., … Karin, M. (2005). Nod2 Mutation in Crohn's Disease Potentiates NF- B Activity and IL-1 Processing. *Science*, *307*(5710), 734-738.

Mähler, M., Bristol, I. J., Leiter, E. H., Workman, A. E., Birkenmeier, E. H., Elson, C. O. et Sundberg, J. P. (1998). Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *274*(3), G544-G551.

Maloy, K. J. et Powrie, F. (2011). Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature*, *474*(7351), 298-306.

Marieb, E. N. et Hoehn, K. (2015). *Human anatomy & physiology*.

Matsuo, K., Ota, H., Akamatsu, T., Sugiyama, A. et Katsuyama, T. (1997). Histochemistry of the surface mucous gel layer of the human colon. *Gut*, *40*(6), 782-789.

McCracken, K. W., Catá, E. M., Crawford, C. M., Sinagoga, K. L., Schumacher, M., Rockich, B. E., … Wells, J. M. (2014). Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. *Nature*, *516*(7531), 400-404.

McGeachy, M. J. et Cua, D. J. (2007). The link between IL-23 and Th17 cell-mediated immune pathologies. *Seminars in Immunology*, *19*(6), 372-376.

McInnes, I. B. et Schett, G. (2007). Cytokines in the pathogenesis of rheumatoid arthritis. *Nature reviews. Immunology*, *7*(6), 429-42.

Meijer, M. J., Mieremet-Ooms, M. A. C., van Duijn, W., van der Zon, A. M., Hanemaaijer, R., Verheijen, J. H., … Verspaget, H. W. (2007). Effect of the anti-tumor necrosis factor-α antibody infliximab on the ex vivo mucosal matrix metalloproteinase–proteolytic phenotype in inflammatory bowel disease. *Inflammatory Bowel Diseases*, *13*(2), 200-210.

Meisel, M., Mayassi, T., Fehlner-Peach, H., Koval, J. C., O'Brien, S. L., Hinterleitner, R., …

Antonopoulos, D. A. (2017). Interleukin-15 promotes intestinal dysbiosis with butyrate deficiency associated with increased susceptibility to colitis. *The ISME journal*, *11*(1), 15-30.

Ménard, D. et Beaulieu, J.-F. (1994). Human Intestinal Brush Border Membrane Hydrolases (p. 319-341). Springer, Boston, MA.

Mention, J.-J., Ben Ahmed, M., Bègue, B., Barbe, U., Verkarre, V., Asnafi, V., … Cerf-Bensussan, N. (2003). Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. *Gastroenterology*, *125*(3), 730-45.

Miyazaki, T., Liu, Z. J., Kawahara, A., Minami, Y., Yamada, K., Tsujimoto, Y., … Taniguchi, T. (1995). Three distinct IL-2 signaling pathways mediated by bcl-2, c-myc, and lck cooperate in hematopoietic cell proliferation. *Cell*, *81*(2), 223-31.

Monteleone, G, Caruso, R., Fina, D., Peluso, I., Gioia, V., Stolfi, C., … Pallone, F. (2006). Control of matrix metalloproteinase production in human intestinal fibroblasts by interleukin 21. *Gut*, *55*(12), 1774-80.

Monteleone, Giovanni, Fantini, M. C., Onali, S., Zorzi, F., Sancesario, G., Bernardini, S., … Pallone, F. (2012). Phase I Clinical Trial of Smad7 Knockdown Using Antisense Oligonucleotide in Patients With Active Crohn's Disease. *Molecular Therapy*, *20*(4), 870-876.

Monteleone, Giovanni, Kumberova, A., Croft, N. M., McKenzie, C., Steer, H. W. et MacDonald, T. T. (2001). Blocking Smad7 restores TGF-β1 signaling in chronic inflammatory bowel disease. *Journal of Clinical Investigation*, *108*(4), 601-609.

Monteleone, Giovanni, Monteleone, I., Fina, D., Vavassori, P., Del Vecchio Blanco, G., Caruso, R., … Pallone, F. (2005). Interleukin-21 enhances T-helper cell type I signaling and interferon-gamma production in Crohn's disease. *Gastroenterology*, *128*(3), 687-94.

Monticelli, L. A., Sonnenberg, G. F., Abt, M. C., Alenghat, T., Ziegler, C. G. K., Doering, T. A., … Artis, D. (2011). Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nature immunology*, *12*(11), 1045-54.

Moolenbeek, C. et Ruitenberg, E. J. (1981). *The « Swiss roll »: a simple technique for histological studies of the rodent intestine*. Laboratory Animals, vol.15.

Moore, K. W., de Waal Malefyt, R., Coffman, R. L. et O'Garra, A. (2001). Interleukin -10 and the Interleukin -10 Receptor. *Annual Review of Immunology*, *19*(1), 683-765.

Mortier, E., Advincula, R., Kim, L., Chmura, S., Barrera, J., Reizis, B., … Ma, A. (2009). Macrophage- and Dendritic-Cell-Derived Interleukin-15 Receptor Alpha Supports Homeostasis of Distinct CD8+ T Cell Subsets. *Immunity*, *31*(5), 811-822.

Mortier, E., Quéméner, A., Vusio, P., Lorenzen, I., Boublik, Y., Grötzinger, J., … Jacques, Y. (2006). Soluble Interleukin-15 Receptor α (IL-15Rα)-sushi as a Selective and Potent Agonist of IL-15 Action through IL-15Rβ/γ. *Journal of Biological Chemistry*, *281*(3), 1612-1619.

Musch, E., Andus, T., Kruis, W., Raedler, A., Spehlmann, M., Schreiber, S., … Feurle, G. E. (2005). Interferon-β-1a for the Treatment of Steroid-Refractory Ulcerative Colitis: A Randomized, Double-Blind, Placebo-Controlled Trial. *Clinical Gastroenterology and Hepatology*, *3*(6), 581-586.

Nakamura, M., Asao, H., Takeshita, T. et Sugamura, K. (1993). Interleukin-2 receptor heterotrimer complex and intracellular signaling. *Seminars in Immunology*, *5*(5), 309-317.

Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., … Sasai, Y. (2012). *No Title*, *10*(6), 771-785.

Natividad, J. M. M., Petit, V., Huang, X., de Palma, G., Jury, J., Sanz, Y., … Verdu, E. F. (2012). Commensal and Probiotic Bacteria Influence Intestinal Barrier Function and Susceptibility to Colitis in Nod1−/−;Nod2−/− Mice. *Inflammatory Bowel Diseases*, *18*(8), 1434-1446.

Nava, P., Koch, S., Laukoetter, M. G., Lee, W. Y., Kolegraff, K., Capaldo, C. T., … Nusrat, A. (2010). Interferon-gamma regulates intestinal epithelial homeostasis through converging beta-catenin signaling pathways. *Immunity*, *32*(3), 392-402.

Nemoto, Y., Kanai, T., Makita, S., Okamoto, R., Totsuka, T., Takeda, K. et Watanabe, M. (2007). Bone Marrow Retaining Colitogenic CD4+ T Cells May Be a Pathogenic Reservoir for Chronic Colitis. *Gastroenterology*, *132*(1), 176-189.

Neurath, M. F., Fuss, I., Kelsall, B. L., Stüber, E. et Strober, W. (1995). Antibodies to interleukin 12 abrogate established experimental colitis in mice. *Journal of Experimental Medicine*, *182*(5), 1281-1290.

Neurath, M F, Weigmann, B., Finotto, S., Glickman, J., Nieuwenhuis, E., Iijima, H., … Blumberg, R. S. (2002). The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. *The Journal of experimental medicine*, *195*(9), 1129-43.

Neurath, Markus F. (2014). Cytokines in inflammatory bowel disease. *Nature Reviews Immunology*, *14*(5), 329-342.

Ng, S. C., Shi, H. Y., Hamidi, N., Underwood, F. E., Tang, W., Benchimol, E. I., … Kaplan, G. G. (2017). Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *The Lancet*, *390*(10114), 2769-2778.

Nielsen, J. et Ji, B. (2015). New insight into the gut microbiome through metagenomics. *Advances in Genomics and Genetics*, *5*, 77.

Nishikawa, J., Kudo, T., Sakata, S., Benno, Y. et Sugiyama, T. (2009). Diversity of mucosa-associated microbiota in active and inactive ulcerative colitis. *Scandinavian Journal of Gastroenterology*, *44*(2), 180-186.

Nishiwaki, T., Ina, K., Goto, H., Watanabe, O., Tsuzuki, T., Furuta, R., … Surgery, G. (2005). Possible involvement of the interleukin-15 and interleukin-15 receptor system in a heightened state of lamina propria B cell activation and. *Journal of Gastroenterology*, *40*(2), 128-136.

Noah, T. K., Donahue, B. et Shroyer, N. F. (2011). Intestinal development and differentiation. *Experimental Cell Research*, *317*(19), 2702-2710.

Noel, G., Baetz, N. W., Staab, J. F., Donowitz, M., Kovbasnjuk, O., Pasetti, M. F. et Zachos, N. C. (2017). A primary human macrophage- enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions. *Nature Publishing Group*, *7*(February), 1-14.

Nozaki, K., Mochizuki, W., Matsumoto, Y., Matsumoto, T., Fukuda, M., Mizutani, T., …

Nakamura, T. (2016). Co-culture with intestinal epithelial organoids allows efficient expansion and motility analysis of intraepithelial lymphocytes. *Journal of Gastroenterology*, *51*(3), 206-213.

O'Connor Jr, W., Kamanaka, M., Booth, C. J., Town, T., Nakae, S., Iwakura, Y., … Flavell, R. A. (2009). A protective function for interleukin 17A in T cell–mediated intestinal inflammation. *Nature Immunology*, *10*(6), 603-609.

Obermeier, F., Hausmann, M., Kellermeier, S., Kiessling, S., Strauch, U. G., Duitman, E., … Rogler, G. (2006). IL-15 protects intestinal epithelial cells. *European Journal of Immunology*, *36*(10), 2691-2699.

Ohri, N., Gerich, M., Fennimore, B. et Kuhn, K. (2016). *Altered colonic intraepithelial lymphocyte composition and function in Crohn's disease*.

Ohta, N., Hiroi, T., Kweon, M.-N., Kinoshita, N., Jang, M. H., Mashimo, T., … Kiyono, H. (2002). IL-15-Dependent Activation-Induced Cell Death-Resistant Th1 Type CD8 +NK1.1+ T Cells for the Development of Small Intestinal Inflammation. *The Journal of Immunology*, *169*(1), 460-468.

Okayasu, I., Hatakeyama, S., Yamada, M., Ohkusa, T., Inagaki, Y. et Nakaya, R. (1990). A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology*, *98*(3), 694-702.

Olsen, S. K., Ota, N., Kishishita, S., Kukimoto-Niino, M., Murayama, K., Uchiyama, H., … Yokoyama, S. (2007). Crystal Structure of the Interleukin-15·Interleukin-15 Receptor α Complex. *Journal of Biological Chemistry*, *282*(51), 37191-37204.

Ordás, I., Eckmann, L., Talamini, M., Baumgart, D. C. et Sandborn, W. J. (2012). Ulcerative colitis. *Lancet*, *380*(9853), 1606-1619.

Overmeer, M., Ponsioen, B., Drost, J., Verissimo, C. S., Mertens, S., Verlaan-klink, I., … Snippert, H. J. (2016). *Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening*, 1-26.

Palamides, P., Jodeleit, H., Föhlinger, M., Beigel, F., Herbach, N., Mueller, T., … Gropp, R. (2016). Characterization and validation of a mouse model for colitis ulcerosa based on NOD-scid IL2R γ^{null} mice reconstituted with peripheral blood mononuclear cells from patients. *Disease Models & Mechanisms*, (August), dmm.025452.

Pandolfi, F., Cianci, R., Pagliari, D., Landolfi, R. et Cammarota, G. (2009). Cellular mediators of inflammation: tregs and TH17 cells in gastrointestinal diseases. *Mediators of inflammation*, *2009*, 132028.

Pearson, J. P. et Brownlee, I. A. (2010). The Interaction of Large Bowel Microflora with the Colonic Mucus Barrier. *International Journal of Inflammation*, *2010*, 1-9.

Perera, P. Y., Lichy, J. H., Waldmann, T. A. et Perera, L. P. (2012). The role of interleukin-15 in inflammation and immune responses to infection: Implications for its therapeutic use. *Microbes and Infection*, *14*(3), 247-261.

Perreault, N. et Beaulieu, J. F. (1996). Use of the dissociating enzyme thermolysin to generate viable human normal intestinal epithelial cell cultures. *Experimental cell research*, *224*(2), 354-64.

Perrier, C., Arijs, I., Staelens, D., Breynaert, C., Cleynen, I., Covens, K., … Ceuppens, J. L.

(2013). Interleukin-15 receptor ?? expression in inflammatory bowel disease patients before and after normalization of inflammation with infliximab. *Immunology*, *138*(1), 47-56.

Peterson, L. W. et Artis, D. (2014). Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nature Reviews Immunology*, *14*(3), 141-153.

Pickert, G., Neufert, C., Leppkes, M., Zheng, Y., Wittkopf, N., Warntjen, M., … Becker, C. (2009). STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *The Journal of Experimental Medicine*, *206*(7), 1465-1472.

Pinto, M., Robineleon, S., Appay, M. D., Kedinger, M., Triadou, N., Dussaulx, E., … Robineloeon, S. (1983, 1 janvier). Enterocyte-like differentiation and polarization of the human-colon carcinoma cell-line Caco-2 in culture.

Powrie, F., Carlino, J., Leach, M. W., Mauze, S. et Coffman, R. L. (1996). A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4+ T cells. *Journal of Experimental Medicine*, *183*(6), 2669-2674.

Powrie, F., Leach, M. W., Mauze, S., Menon, S., Caddle, L. B. et Coffman, R. L. (1994). Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity*, *1*(7), 553-62.

Rakoff-Nahoum, S., Pglino, J., Eslami-Varzaneh, F., Edberg, S. et Medzhitov, R. (2004). Recognition of comensal microflora by toll-like receptors in required for intestinal homeostasis. *Cell*, *118*, 229-241.

Ramanathan, S., Gagnon, J., Leblanc, C., Rottapel, R. et Ilangumaran, S. (2006). Suppressor of Cytokine Signaling 1 Stringently Regulates Distinct Functions of IL-7 and IL-15 In Vivo during T Lymphocyte Development and Homeostasis. *The Journal of Immunology*, *176*(7), 4029-4041.

Rani, R., Smulian, A. G., Greaves, D. R., Hogan, S. P. et Herbert, D. R. (2011). TGF-β limits IL-33 production and promotes the resolution of colitis through regulation of macrophage function. *European Journal of Immunology*, *41*(7), 2000-2009.

Ratthé, C. et Girard, D. (2004). Interleukin-15 enhances human neutrophil phagocytosis by a Syk-dependent mechanism: importance of the IL-15Rα chain. *Journal of Leukocyte Biology*, *76*(1), 162-168.

Reese, T. A., Bi, K., Kambal, A., Filali-Mouhim, A., Beura, L. K., Bürger, M. C., … Virgin, H. W. (2016). Sequential Infection with Common Pathogens Promotes Human-like Immune Gene Expression and Altered Vaccine Response. *Cell Host & Microbe*, *19*(5), 713-719.

Regamey, N., Obregon, C., Ferrari-Lacraz, S., van Leer, C., Chanson, M., Nicod, L. P. et Geiser, T. (2007). Airway Epithelial IL-15 Transforms Monocytes into Dendritic Cells. *American Journal of Respiratory Cell and Molecular Biology*, *37*(1), 75-84.

Reinecker, H.-C., MacDermott, R. P., Mirau, S., Dignass, A. et Podolsky, D. K. (1996). Intestinal epithelial cells both express and respond to interleukin 15. *Gastroenterology*, *111*(6), 1706-1713.

Reinisch, W., Hommes, D. W., Van Assche, G., Colombel, J.-F., Gendre, J.-P., Oldenburg, B., … Pearce, T. (2006). A dose escalating, placebo controlled, double blind, single dose and multidose, safety and tolerability study of fontolizumab, a humanised anti-interferon gamma antibody, in patients with moderate to severe Crohn's disease. *Gut*, *55*(8), 1138-44.

Rentzos, M., Cambouri, C., Rombos, A., Nikolaou, C., Anagnostouli, M., Tsoutsou, A., … Vassilopoulos, D. (2005). *IL-15 is elevated in serum and cerebrospinal fluid of patients with multiple sclerosis*.

Rimoldi, M., Chieppa, M., Salucci, V., Avogadri, F., Sonzogni, A., Sampietro, G. M., … Rescigno, M. (2005). Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nature Immunology*, *6*(5), 507-514.

Ring, A. M., Lin, J.-X., Feng, D., Mitra, S., Rickert, M., Bowman, G. R., … Garcia, K. C. (2012). Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. *Nature Immunology*, *13*(12), 1187-1195.

Rochman, Y., Spolski, R. et Leonard, W. J. (2009). New insights into the regulation of T cells by γ c family cytokines. *Nature Publishing Group*, *9*(7), 480-490.

Rolhion, N. et Chassaing, B. (2016). When pathogenic bacteria meet the intestinal microbiota. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1707), 20150504.

Rousset, M. (1986). The human colon carcinoma cell lines HT-29 and Caco-2: two in vitro models for the study of intestinal differentiation. *Biochimie*, *68*(9), 1035-40.

Ruemmele, F. M., Gurbindo, C., Mansour, A. M., Marchand, R., Levy, E. et Seidman, E. G. (1998). Effects of Interferon γ on growth, apoptosis, and MHC class II expression of immature rat intestinal crypt (IEC-6) cells. *Journal of Cellular Physiology*, *176*(1), 120-126.

Sakai, T., Kusugami, K., Nishimura, H., Ando, T., Yamaguchi, T., Ohsuga, M., … Yoshikai, Y. (1998). Interleukin 15 activity in the rectal mucose of inflammatory bowel disease. *Gastroenterology*, *114*, 1237-1243.

Sakuraba, A., Sato, T., Kamada, N., Kitazume, M., Sugita, A. et Hibi, T. (2009). Th1/Th17 Immune Response Is Induced by Mesenteric Lymph Node Dendritic Cells in Crohn's Disease. *Gastroenterology*, *137*(5), 1736-1745.

Sambasivarao, S. V. (2013). Dextran Sulfate Sodium (DSS)-Induced Colitis in Mice. *NIH Public Access*, *18*, 1199-1216.

Sartor, R. B. (2006). Mechanisms of Disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nature Clinical Practice Gastroenterology & Hepatology*, *3*(7), 390-407.

Sathaliyawala, T., Kubota, M., Yudanin, N., Turner, D., Camp, P., Thome, J. J. C., … Farber, D. L. (2013). Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. *Immunity*, *38*(1), 187-97.

Sato, T., Stange, D. E., Ferrante, M., Vries, R. G. J., Van Es, J. H., Van Den Brink, S., … Clevers, H. (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology*, *141*(5), 1762-1772.

Sato, T., van Es, J. H., Snippert, H. J., Stange, D. E., Vries, R. G., van den Born, M., … Clevers, H. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature*, *469*(7330), 415-418.

Sato, T., Vries, R. G., Snippert, H. J., Wetering, M. Van De, Barker, N., Stange, D. E., … Clevers, H. (2009). Single Lgr5 stem cells build crypt – villus structures in vitro without a mesenchymal niche. *Nature*, *459*(7244), 262-265.

Schmitz, H., Barmeyer, C., Fromm, M., Runkel, N., Foss, H. D., Bentzel, C. J., … Schulzke, J.

D. (1999). Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. *Gastroenterology*, *116*(2), 301-9.

Sharon, M., Klausner, R. D., Cullen, B. R., Chizzonite, R. et Leonard, W. J. (1986). Novel interleukin-2 receptor subunit detected by cross-linking under high-affinity conditions. *Science (New York, N.Y.)*, *234*(4778), 859-63.

Shen, X., Shi, R., Zhang, H., Li, K., Zhao, Y. et Zhang, R. (2010). The Toll-Like Receptor 4 D299G and T399I Polymorphisms Are Associated with Crohn's Disease and Ulcerative Colitis: A Meta-Analysis. *Digestion*, *81*(2), 69-77.

Shulzhenko, N., Morgun, A., Hsiao, W., Battle, M., Yao, M., Gavrilova, O., … Matzinger, P. (2011). Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. *Nature Medicine*, *17*(12), 1585-1593.

Siakavellas, S. I. et Bamias, G. (2012). Role of the IL-23/IL-17 axis in Crohn's disease. *Discovery medicine*, *14*(77), 253-62.

Silva, J. P. B., Navegantes-Lima, K. C., de Oliveira, A. L. B., Rodrigues, D. V. S., Gaspar, S. L. F., Monteiro, V. V. S., … Monteiro, M. C. (2018). Protective Mechanisms of Butyrate on Inflammatory Bowel Disease. *Current Pharmaceutical Design*, *24*(35), 4154-4166.

Skov, S., Bonyhadi, M., Odum, N. et Ledbetter, J. A. (2000). IL-2 and IL-15 Regulate CD154 Expression on Activated CD4 T Cells. *The Journal of Immunology*, *164*(7), 3500-3505.

Smith, M. E. et Morton, D. G. (2010). *The digestive system : basic edition science and clinical conditions*. Churchill Livingstone.

Spits, H. et Cupedo, T. (2012). Innate Lymphoid Cells: Emerging Insights in Development, Lineage Relationships, and Function. *Annual Review of Immunology*, *30*(1), 647-675.

Steel, J. C., Ramlogan, C. A., Yu, P., Sakai, Y., Forni, G., Waldmann, T. A. et Morris, J. C. (2010). *Interleukin-15 and Its Receptor Augment Dendritic Cell Vaccination against the neu Oncogene through the Induction of Antibodies Partially Independent of CD4 Help*, *70*(3).

Stein, J., Hartmann, F. et Dignass, A. U. (2010). Diagnosis and management of iron deficiency anemia in patients with IBD. *Nature Reviews Gastroenterology & Hepatology*, *7*(11), 599-610.

Stonier, S. W. et Schluns, K. S. (2010). Trans-presentation: A novel mechanism regulating IL-15 delivery and responses. *Immunology Letters*, *127*(2), 85-92.

Strober, W., Fuss, I. J. et Blumberg, R. S. (2002). The immunology of mucosal models of inflammation. *Annual review of immunology*, *20*(1), 495-549.

Su, L., Nalle, S. C., Shen, L., Turner, E. S., Singh, G., Breskin, L. A., … Turner, J. R. (2013). TNFR2 Activates MLCK-Dependent Tight Junction Dysregulation to Cause Apoptosis-Mediated Barrier Loss and Experimental Colitis. *Gastroenterology*, *145*(2), 407-415.

Sun, G., Mackey, L. V., Coy, D. H., Yu, C.-Y. et Sun, L. (2015). The Histone Deacetylase Inhibitor Vaproic Acid Induces Cell Growth Arrest in Hepatocellular Carcinoma Cells via Suppressing Notch Signaling. *Journal of Cancer*, *6*(10), 996-1004.

Suzuki, K., Meek, B., Doi, Y., Muramatsu, M., Chiba, T., Honjo, T. et Fagarasan, S. (2004). Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proceedings of the National Academy of Sciences*, *101*(7), 1981-1986.

Swidsinski, A., Ladhoff, A., Pernthaler, A., Swidsinski, S., Loening-Baucke, V., Ortner, M., … Lochs, H. (2002). Mucosal flora in inflammatory bowel disease. *Gastroenterology*, *122*(1), 44-54.

Szabo, S. J., Sullivan, B. M., Stemmann, C., Satoskar, A. R., Sleckman, B. P. et Glimcher, L. H. (2002). Distinct Effects of T-bet in TH1 Lineage Commitment and IFN-gamma Production in CD4 and CD8 T Cells. *Science*, *295*(5553), 338-342.

Tagaya, Y., Kurys, G., Thies, T. A., Losi, J. M., Azimi, N., Hanover, J. A., … Waldmann, T. A. (1997). Generation of secretable and nonsecretable interleukin 15 isoforms through alternate usage of signal peptides. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(26), 14444-9.

Tait Wojno, E. D. et Artis, D. (2012). Innate Lymphoid Cells: Balancing Immunity, Inflammation, and Tissue Repair in the Intestine. *Cell Host & Microbe*, *12*(4), 445-457.

Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., … Sugamura, K. (1992). Cloning of the gamma chain of the human IL-2 receptor. *Science (New York, N.Y.)*, *257*(5068), 379-82.

Takeshita, T., Ohtani, K., Asao, H., Kumaki, S., Nakamura, M. et Sugamura, K. (1992). An associated molecule, p64, with IL-2 receptor beta chain. Its possible involvement in the formation of the functional intermediate-affinity IL-2 receptor complex. *Journal of immunology (Baltimore, Md. : 1950)*, *148*(7), 2154-8.

Tanaka, T., Kitamura, F., Nagasaka, Y., Kuida, K., Suwa, H. et Miyasaka, M. (1993). Selective long-term elimination of natural killer cells in vivo by an anti-interleukin 2 receptor beta chain monoclonal antibody in mice. *Journal of Experimental Medicine*, *178*(3), 1103-1107.

Tanaka, Y., Morita, C. T., Tanaka, Y., Nieves, E., Brenner, M. B. et Bloom, B. R. (1995). Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. *Nature*, *375*(6527), 155-8.

Tilg, H., Ulmer, H., Kaser, A. et Weiss, G. (2002). Role of IL-10 for Induction of Anemia During Inflammation. *The Journal of Immunology*, *169*(4), 2204-2209.

Tomasello, E. et Bedoui, S. (2013). Intestinal innate immune cells in gut homeostasis and immunosurveillance. *Immunology and Cell Biology*, *91*(3), 201-203.

Tsudo, M., Kozak, R. W., Goldman, C. K. et Waldmann, T. A. (1987). Contribution of a p75 interleukin 2 binding peptide to a high-affinity interleukin 2 receptor complex. *Proceedings of the National Academy of Sciences*, *84*(12), 4215-4218.

Uhlin, M., Sandalova, E., Masucci, M. G. et Levitsky, V. (2005). Help signals provided by lymphokines modulate the activation and apoptotic programs induced by partially agonistic peptides in specific cytotoxic T lymphocytes. *European Journal of Immunology*, *35*(10), 2929-2939.

Ungar, B. et Kopylov, U. (2016). Advances in the development of new biologics in inflammatory bowel disease. *Annals of Gastroenterology*, *29*(3), 243-8.

Vachon, P. H. et Beaulieu, J. F. (1992). Transient mosaic patterns of morphological and functional differentiation in the Caco-2 cell line. *Gastroenterology*, *103*(2), 414-23.

Vainer, B., Nielsen, O. H., Hendel, J., Horn, T. et Kirman, I. (2000). Colonic expression and synthesis of interleukin 13 and interleukin 15 in inflammatory bowel disease. *Cytokine*,

12(10), 1531-1536.

van Ampting, M. T. J., Loonen, L. M. P., Schonewille, A. J., Konings, I., Vink, C., Iovanna, J., … Bovee-Oudenhoven, I. M. J. (2012). Intestinally secreted C-type lectin Reg3b attenuates salmonellosis but not listeriosis in mice. *Infection and immunity*, *80*(3), 1115-20.

Van Belle, T. et Grooten, J. (2005). IL-15 and IL-15Ralpha in CD4+T cell immunity. *Archivum immunologiae et therapiae experimentalis*, *53*(2), 115-26.

Van den Brande, J. M. H., Braat, H., van den Brink, G. R., Versteeg, H. H., Bauer, C. A., Hoedemaeker, I., … van Deventer, S. J. H. (2003). Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology*, *124*(7), 1774-85.

van der Flier, L. G. et Clevers, H. (2009). Stem Cells, Self-Renewal, and Differentiation in the Intestinal Epithelium. *Annual Review of Physiology*, *71*(1), 241-260.

Van der Sluis, M., De Koning, B. A. E., De Bruijn, A. C. J. M., Velcich, A., Meijerink, J. P. P., Van Goudoever, J. B., … Einerhand, A. W. C. (2006). Muc2-Deficient Mice Spontaneously Develop Colitis, Indicating That MUC2 Is Critical for Colonic Protection. *Gastroenterology*, *131*(1), 117-129.

van Heel, D. A. (2006). Interleukin 15: its role in intestinal inflammation. *Gut*, *55*(4), 444-445.

Varol, C., Landsman, L., Fogg, D. K., Greenshtein, L., Gildor, B., Margalit, R., … Jung, S. (2007). Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *The Journal of Experimental Medicine*, *204*(1), 171-180.

Velcich, A., Yang, W., Heyer, J., Fragale, A., Nicholas, C., Viani, S., … Augenlicht, L. (2002). Colorectal Cancer in Mice Genetically Deficient in the Mucin Muc2. *Science*, *295*(5560), 1726-1729.

Waldmann, T. A. et Tagaya, Y. (1999). The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annual Review of Immunology*, *17*(1), 19-49.

Waldmann, Thomas A. (2006, août). The biology of interleukin-2 and interleukin-15: Implications for cancer therapy and vaccine design (vol. 6, p. 595-601).

Waldmann, Thomas A. (2004). Targeting the interleukin-15/interleukin-15 receptor system in inflammatory autoimmune diseases. *Arthritis research & therapy*, *6*(4), 174-7.

Weldon, L., Abolins, S., Lenzi, L., Bourne, C., Riley, E. M. et Viney, M. (2015). The Gut Microbiota of Wild Mice. *PLOS ONE*, *10*(8), e0134643.

Wetering, M. Van De, Francies, H. E., Garnett, M. J., Wetering, M. Van De, Francies, H. E., Francis, J. M., … Saez-rodriguez, J. (2015). Prospective Derivation of a Living Organoid Biobank of Colorectal Cancer Patients Resource Prospective Derivation of a Living Organoid Biobank of Colorectal Cancer Patients. *Cell*, *161*(4), 933-945.

Wilson, C. L., Ouellette, A. J., Satchell, D. P., Ayabe, T., López-Boado, Y. S., Stratman, J. L., … Parks, W. C. (1999). Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science (New York, N.Y.)*, *286*(5437), 113-7.

Wirtz, S., Finotto, S., Kanzler, S., Lohse, A. W., Blessing, M., Lehr, H. A., … Neurath, M. F. (1999). Cutting edge: chronic intestinal inflammation in STAT-4 transgenic mice:

characterization of disease and adoptive transfer by TNF- plus IFN-gamma-producing CD4+ T cells that respond to bacterial antigens. *Journal of immunology (Baltimore, Md. : 1950)*, *162*(4), 1884-8.

Wu, T.-S., Lee, J.-M., Lai, Y.-G., Hsu, J.-C., Tsai, C.-Y., Lee, Y.-H. et Liao, N.-S. (2002). Reduced expression of Bcl-2 in CD8+ T cells deficient in the IL-15 receptor alpha-chain. *Journal of immunology (Baltimore, Md. : 1950)*, *168*(2), 705-12.

Wynn, T. A. et Vannella, K. M. (2016). Macrophages in tissue repair, regeneration, and fibrosis. *Immunity*, *44*(3), 450.

Xavier, R. J. et Podolsky, D. K. (2007). Unravelling the pathogenesis of inflammatory bowel disease. *Nature*, *448*(7152), 427-434.

Yamada, M., Ohkusa, T. et Okayasu, I. (1992). Occurrence of dysplasia and adenocarcinoma after experimental chronic ulcerative colitis in hamsters induced by dextran sulphate sodium. *Gut*, *33*(11), 1521-7.

Yamamoto, M. et Matsumoto, S. (2016). Gut microbiota and colorectal cancer. *Genes and Environment*, *38*(1), 11.

Yokoyama, S., Watanabe, N., Sato, N., Perera, P., Filkoski, L., Tanaka, T., … Perera, L. P. (2009). Antibody-mediated blockade of IL-15 reverses the autoimmune intestinal damage in transgenic mice that overexpress IL-15 in enterocytes. *Proceedings of the National Academy of Sciences*, *106*(37), 15849-15854.

Young, B. (Pathologist). (2006). *Wheater's functional histology : a text and colour atlas*. (s. l.) : Churchill Livingstone/Elsevier.

Yu, Q., Tang, C., Xun, S., Yajima, T., Takeda, K. et Yoshikai, Y. (2006). MyD88-dependent signaling for IL-15 production plays an important role in maintenance of CD8 alpha alpha TCR alpha beta and TCR gamma delta intestinal intraepithelial lymphocytes. *Journal of immunology (Baltimore, Md. : 1950)*, *176*(10), 6180-5.

Yuan, X., Dong, Y., Tsurushita, N., Tso, J. Y. et Fu, W. (2018). CD122 blockade restores immunological tolerance in autoimmune type 1 diabetes via multiple mechanisms. *JCI insight*, *3*(2).

Zeuthen, L. H., Fink, L. N. et Frokiaer, H. (2007). Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-? *Immunology*, *0*(0), 070726024918001-???

Zhang, F. X., Kirschning, C. J., Mancinelli, R., Xu, X.-P., Jin, Y., Faure, E., … Arditi, M. (1999). Bacterial Lipopolysaccharide Activates Nuclear Factor-κB through Interleukin-1 Signaling Mediators in Cultured Human Dermal Endothelial Cells and Mononuclear Phagocytes. *Journal of Biological Chemistry*, *274*(12), 7611-7614.

Zhao, H., Nguyen, H. et Kang, J. (2005). Interleukin 15 controls the generation of the restricted T cell receptor repertoire of gamma delta intestinal intraepithelial lymphocytes. *Nature immunology*, *6*(12), 1263-71.

Zhu, X., Suen, K. L., Barbacid, M., Bolen, J. B. et Fargnoli, J. (1994). Interleukin-2-induced tyrosine phosphorylation of Shc proteins correlates with factor-dependent T cell proliferation. *The Journal of biological chemistry*, *269*(8), 5518-22.

9. ANNEXES

Annex 1: License for the use of Figure 2.

SPRINGER NATURE LICENSE
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