Review article: distinctions between ileal and colonic Crohn's disease: from physiology to pathology

Running title: ileal and colonic Crohn's disease differences

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Summary

Background: Ileal and colonic Crohn's disease seem to be two separate entities.

Aims: To describe the main physiological distinctions between the small and the large intestine and to analyse the differences between ileal and colonic Crohn's disease.

Methods: The relevant literature was critically examined and synthesised.

Results: In physiological situation, the small and the large intestine present fundamental distinctions (anatomy, cellular populations, immune defence, microbiota). The differences between ileal and colonic Crohn's disease are highlighted by heterogeneous body of evidence including clinical features (natural history of the disease, efficacy of treatments and monitoring), epidemiological data (smoking status, age, gender) and biological data (genetics, microbiota, immunity, mesenteric fat). However, the contribution of these factors to disease location remains poorly understood.

Conclusion: The classification of ileal and colonic Crohn's disease as distinct subphenotypes is well supported by the literature. The comprehension of these differences could be exploited to develop more individualised patient care.

Keywords: Crohn's disease, disease location, ileum, colon

Introduction

Crohn's disease (CD) is characterised by relapsing-remitting phases related to transient inflammatory flares. Contrary to ulcerative colitis (UC), the other inflammatory bowel disease (IBD), inflammation in CD can be transmural and can affect all the gastrointestinal tract. However, CD is most frequently located in the ileum and/or the colon[1].

Although the introduction of anti-tumor necrosis factor-α (TNFα) antibodies and other biologic treatments revolutionised the management of CD patients, new drugs are necessary for patients exhibiting a primary (10-30%) or a secondary (23-46%) non-response to biologic treatments[2]. Furthermore, the prediction of clinical outcomes and the non-invasive monitoring of disease activity are still unmet clinical needs. These observations together with the heterogeneous presentation of CD plead for the development of more personalised approaches[3]. In this context, disease location appears as a simple way by which patients could be stratified and next beneficiate of better fitted therapy and monitoring. Indeed, ileal and colonic CD present distinct features that might be exploited to better individualise the management of patients[4,5].

In 2001, it was showed for the first time that disease location, contrary to disease behaviour, remains relatively stable during the natural history of CD[6]. This observation highly suggested the presence of genetic factors influencing disease location[6], this assumption was thereafter confirmed. Single nucleotide polymorphisms (SNPs) have been associated with ileal (intermediate conductance calcium-activated potassium channel protein 4: *KCNN4*; leucine-rich repeat kinase 2: *LRRK2*; nucleotide-binding oligomerization domain-containing 2: *NOD2*; transcription factor 4: *TCF4*; low-density lipoprotein receptor-related protein 6: *LRP6*; autophagy-related 16-like gene: *ATG16L1*) or colonic CD (major histocompatibility complex: *MHC*)[1,7–12]. The influence of genetics on disease location is also supported by a genetic risk score (including known risk loci for IBD) which situated ileocolonic CD between

ileal and colonic CD[1]. However, CD is a complex disorder where genetics is only one piece of a complex puzzle. Currently, a heterogeneous body of evidence supports the existence of distinct pathological processes between ileal and colonic CD. By integrating and synthesising these findings, the present review aims to provide a large overview of the topic. As a prerequisite, we first describe the main constitutive factors (anatomy, cellular populations, immune defence, microbiota) distinguishing the small from the large intestine. A graphical summary of this section is presented in Figure 1.

1-Main physiological features distinguishing the small from the large intestine

1.1-General considerations

At the anatomical level, the intestinal epithelium is relatively flat in the colon while it presents luminal projections in the ileum due to the finger-like villi[13]. In addition, the apical protrusions of epithelial cells, namely microvilli, constitute a particularity of the small intestine promoting nutrient absorption. In a schematic manner, nutrients are absorbed in the small intestine while the large intestine is involved in fermentation and water absorption.

The gut represents the most important interface of the body with the external world, it is thus highly exposed to microorganisms. Complex cellular processes exclude pathogens from the intestinal mucosa while others allow their entry, a fundamental mechanism promoting immune system maturation and tolerance. Microbiota and host co-evolved, establishing host-commensal, host-symbiotic and host-parasite relationships. The host-microbial interactions are intensively negotiated and lead to a tight homeostatic control of the gut barrier. The microbial composition influences the host immune response, thus generating a feedback that in turn shapes the microbiota. These complex interactions evolved in distinct spaces of the gastro-intestinal tract, and led to gut segment-specific relations between host and microbiota. Besides, the small and the large intestine are recognised as two distinct immunological sites[14].

1.2-Epithelium

The cellular composition of the small and the large intestine epithelium exhibits some specificities. Compared to the large intestine, the small intestine epithelium is characterised by the presence of Paneth cells in the crypt and a higher number of M cells[14]. The Paneth cells are specialised in the secretion of anti-microbial peptides (AMPs) while the M cells are involved in the transport and presentation of luminal antigens to immune cells[14]. Due to distinct intestinal epithelial cells (IECs) population and gene expression profiles, the secreted AMPs present specificities in the small and the large intestine. The small intestine epithelium is characterised by the secretion of α-defensins/lysozyme/phospholipase A2 (Paneth cells) and regenerating islet derived protein-γ (REG3γ) (Paneth cells and enterocytes)[15]. The large intestine epithelium is characterised by the secretion of β-defensins and cathelicidins by enterocytes[15]. Compared to the small intestine, the large intestine epithelium presents a higher number of Goblet cells which are specialised in the secretion of mucus. In the small intestine, mucus is organised in a single layer firmly attached to the epithelium whereas in the large intestine, mucus is composed of two layers: a loose layer (outer) overlapping a dense layer (inner) attached to the epithelium[16]. The IECs are also composed of enteroendocrine cells (<1%)[17]. These cells show a higher frequency in the small intestine and the rectum than the colon[18]. In addition, enteroendocrine cells present distinct morphology and hormone secretion profiles in the small and the large intestine[18]. As the cellular composition of the differentiated epithelial cells varies in relation to gut segment, it is not surprising to find distinctions in the progenitor cells. In human, stem cells from the small and the large intestine showed distinct cell surface markers, molecular signatures and response to differentiation signals[19].

1.3-Lamina propria

Throughout the gastrointestinal tract, immune cells mainly reside in the lamina propria and their density is higher in the small than the large intestine[14].

Data from mice showed that dendritic cells (DCs) are present in a much higher number in the small than the large intestine[14], they migrate in anatomically distinct lymph nodes called small intestinal mesenteric lymph node (sMLN) or colonic MLN (cMLN)[20]. Such segregation is associated with separate antigen migration and different mechanisms of naive T-cell priming[20]. In the intestinal lamina propria of human and mice, DCs subsets are grouped into type 1 DC (DC1) and type 2 DC (DC2) which differ in their functions and surface markers[21]. In mice, the DC2 ($CD103^+CD11b^+$) predominates in the small intestine while this is the DC1 ($CD103^+CD11b^-$) in the large intestine[21]. Similar results were reported in human (DC2: $CD103^+Sirp\alpha^+$; DC1: $CD103^+Sirp\alpha^-$)[22]. In mouse models, DC1 and DC2 are associated with key functional distinctions between the small and the large intestine. Whereas the DC2 drives Th17 response trough the transcription factor interferon regulatory factor 4 (IRF4), the DC1 stimulates the Th1 response via IRF8[23,24]. In line with these observations, in mice the proportion and absolute number of $CD4^+$ T cells with a Th17 phenotype is higher in the small than the large intestine[25,26] and, it has been shown that the Th17 response is restricted to the ileum upon bacterial colonisation[27].

Between the small and the large intestine, distinct mechanisms of tolerance are also suspected due to differences in regulatory T cell populations[14]. Indeed, in mice Tr1 (forkhead box $P3^-$, $Foxp3^-$) regulatory T cells predominate in the small intestine while this is the natural ($Foxp3^+$) regulatory T cells in the large intestine[28].

In the lamina propria and the submucosa of the small and the large intestine, eosinophils are present in small number. Intriguingly, the inhibitory receptor of B cells, CD22, is highly expressed in the eosinophils of the small (jejunum>duodenum>ileum) but not the large intestine of mice[29].

Regarding plasmacytoid DCs, macrophages, mast cells, basophils and natural killer cells, no clear differences in term of population or expression patterns have been reported between the small and the large intestine (data from mice)[14]. Neutrophils are a special case since their presence in the intestinal mucosa is mainly related to a pathological situation, they are scarce or even absent in a healthy gut. Thus, neutrophils will be discussed in the context of CD (see part 2.4).

1.4-The gut-associated lymphoid tissue (GALT)

The GALT encompasses different structures and cells such as Peyer's patches, isolated lymphoid follicles, cryptopatches and intraepithelial lymphocytes (IELs)[30]. The Peyer's patches are particular immune sites present in the lamina propria of the ileum, they consist of aggregated lymphoid nodules (mainly composed of B and T cells) covered by M cells at the apical side. In the intestinal mucosa, the largest number of B cells is found in the Peyer's patches and these cells are notably specialised in the secretion of immunoglobulin A (IgA) which, through binding with the polymeric immunoglobulin receptor, are transported across epithelial cells and then secreted in the intestinal lumen[14,31]. Hence, IgA secretion constitutes a first line defence against pathogen infiltration which is more present in the small than the large intestine. In mice, Peyer's patches contain particular DC subsets, the $CD8\alpha^{+}CD11b^{-}$ (interfollicular region) and the $CD8\alpha^{-}CD11b^{+}$ (subepithelial dome)[21]. In human and mice Peyer's patches, the lysozyme-expressing dendritic cells (LysoDCs) is a unique DCs subset able to synthesize lysozyme[32,33]. These cells are functionally characterised by a high capacity of antigen sampling and a high phagocytic activity against dead cells (including M cells)[33].

In mice, specific immune mechanisms related to gut segments have been identified through the study of the lymphoid tissue-inducer cells expressing the natural killer receptor (LTi NKR cells), a subpopulation of innate lymphoid cells present in the GALT[34]. The transcription

factor RAR-related orphan receptor-γ (RORγ) is more frequently expressed in LTi NKR cells of the small than those of the large intestine[35]. In the presence or absence of RORγ, LTi NKR cells produce respectively interleukin-22 (IL-22) or interferon gamma (IFNγ)[35]. This observation implies different immune mechanisms between the small and the large intestine since IL-22 stimulates the epithelial defences (e.g., AMPs and mucins) while IFNγ is well known to promote Th1 differentiation[36,37].

The IELs are intercalated between epithelial cells and, in mice, they show a higher density (ratio of IELs to enterocytes) in the small than the large intestine[38]. Based on their T cell receptor types ($\alpha\beta^+$ or $\gamma\delta^+$) and their expression of CD3, CD4 and CD8, IELs subsets differ between the small and the large intestine (data from human and mice)[38,39]. In addition, it is well established that, compared to the large intestine, the small intestine exhibits a lower proportion of naive IELs and a higher proportion of activated/memory IELs (data from mice)[39].

1.5-Microbiota

Between the small and the large intestine, the quantity and the composition of the microbiota present also particularities. One millilitre of human intestinal content contains 10^3-10^5 (duodenum-jejunum), 10^8 (ileum) and 10^{10}-10^{11} (colon) bacteria[40,41]. In human and mice, the dominant bacterial families of the small intestine are the *Lactobacillaceae* and the *Enterobacteriaceae*; while in the large intestine this is the *Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae* and *Ruminococcaceae*[16,41].

The mucus layers of each gut segment offer protected niches for particular populations of bacteria namely "mucus-associated microorganisms"[42]. By degrading mucins, *Akkermansia muciniphila* and *Bacteroides fragilis* are well adapted to the mucus layers of the colon where they are found enriched in mice and humans[16,42]. More precisely, *Akkermansia muciniphila*

resides in the outer mucus layer while *Bacteroides fragilis* is present in both the outer and inner mucus layers including crypts[16,42,43]. In mice, segmented filamentous bacteria are well-known to colonise the mucus layer of the ileum where they attach to the epithelium while colonic outer mucus layer is enriched in bacteria such as *Bacteroides acidifaciens* which is a mucin-degrading bacteria[16,42,44].

The composition of microbiota is largely influenced by physiological gradients along the gastro-intestinal tract. Indeed, pH increases while oxygen, antimicrobial peptides and mucus thickness decrease from the small to the large intestine[16,42].

2-Distinctions between ileal and colonic Crohn's disease

This part is summarised in the Table 1.

2.1-Dysfunction of Paneth cells in ileal Crohn's disease: where genetic factors converge?

A dysfunction of Paneth cells in ileal CD is supported by the study of genetic variants affecting *NOD2, LRRK2, TCF4, LRP6, ATG16L1*, X-box binding protein 1 (*XBP1*) and *KCNN4*. Except *XBP1*, all these genetic variants are associated with a higher risk to develop an ileal CD[7–12].

In CD patients carrying *NOD2* or *ATG16L1* genetic variants, abnormal Paneth cell morphology has been observed through histological analysis of the lysozyme granules[45,46]. These results have been corroborated in a mice model and intestinal organoid culture. The defect of autophagy in mice hypomorphic for *Atg16l1* perturbed the secretion of lysozyme by Paneth cells[45]. In mice, the culture of intestinal organoid demonstrated that, in Paneth cells, NOD2 and LRRK2 are part of a pathway orchestrating the exocytosis of the lysozyme-containing granules[47].

The SNPs affecting *TCF4*, *LRP6* and *KCNN4* have been associated with ileal CD and proteins coded by these genes are involved in Paneth cell maturation (via the Wnt pathway) and secretion[8,9,11]. Hence, it was suspected that *TCF4*, *LRP6* and *KCNN4* polymorphisms could induce Paneth cell dysfunction[8,9,11]. However, this mechanism remains to be proven by functional experiments.

The role of endoplasmic reticulum (ER) stress in Paneth cell dysfunction has been highlighted by studying *XBP1*. Indeed, this protein is a transcription factor implicated in the rescue of ER stress and its deletion in mice caused apoptotic death of Paneth cells and spontaneous enteritis[48]. In another study, a mice model with Paneth cell-specific deletion of *Xbp1* has been generated[49]. A majority of those mice (75%) developed spontaneous enteritis, their Paneth cells presented ER stress, autophagy and abnormal lysozyme granules[49]. However, the link between *XBP1* mutations, Paneth cell dysfunction and disease location has only been shown in mice models. In human, *XBP1* risk variants for CD have not been associated with ileal CD. Thus, mice models and human data are not well in agreement to show a role of *XPB1* polymorphisms in disease location.

In CD, much attention has been paid to the relation between genetic variants and Paneth cell functions. However, the incriminated mutations could affect other cell types. In addition to be expressed by Paneth cells, NOD2 is found in macrophages, dendritic cells, goblet cells, intestinal stem cells and enterocytes[50,51]. On the other hand, ER stress, autophagy and the Wnt pathway are ubiquitous. More research is needed to characterise the functional consequences of the genetic variants associated with ileal CD.

2.2-Higher disruption of the microbiota in ileal than colonic Crohn's disease

At the interplay between genetic and environmental factors, microbiota could be a key determinant of disease location in CD. IBD patients present a dysbiotic intestinal flora

characterised by a reduction of bacterial diversity (particularly the Firmicutes phylum)[52]. However, such a well-recognised feature of IBD appears to be specific to ileal CD. In a general manner, the microbiota of patients with isolated colonic CD seems close to healthy individuals while patients with ileal CD present a clear disruption of the intestinal flora[53,54]. Compared to healthy individuals, the diversity of bacteria in stools is diminished in ileal but not colonic CD[53,55]. Overall, ileal CD is characterised by a reduction of Firmicutes and an increase of Proteobacteria. In contrast to patients with a predominant colonic CD, patients with a predominant ileal CD showed a reduction of *Faecalibacterium prausnitzii* (Firmicutes phylum) and *Roseburia* (Firmicutes phylum) in their stools when compared to healthy individuals[53]. In the mucosa, similar results were reported for *F. prausnitzii*[56]. Besides, a reduction of *F. prausnitzii* in the ileal mucosa (surgical resection for active disease) of CD patients has been associated with a higher risk of endoscopic recurrence[57]. Given that *F. prausnitzii* presents anti-inflammatory properties, this could explain the inverse relation between abundance of this bacteria and CD activity[57]. On the other hand, the ileal mucosa of patients with ileal CD showed a higher level of *Escherichia coli* (Proteobacteria phylum) than the ileal mucosa of patients with isolated colonic CD and healthy individuals[58]. In this study, the identified *E. coli* strains were specifically harboured in the ileum and their number was positively correlated with endoscopic (Crohn's disease endoscopic index score: CDEIS) and histologic score of disease activity. The increase of *E. coli* in ileal CD has been confirmed[56]. Furthermore, adherent-invasive *E. coli* (AIEC) is almost exclusively associated with the ileal form of CD[59]. In addition to adhere and invade the epithelium, this bacteria strain is able to replicate inside macrophages and to stimulate an inflammatory response. The AIEC also showed the capacity to translocate across the M cells and to interact with the Peyer’s patches[60]. These mechanisms could explain the link between AIEC and ileal CD[60].

2.3- Fibrosis and creeping fat are primarily found in ileal Crohn's disease

Fibrosis is a complex complication of CD for which no specific treatment exists[61]. A higher rate of fibrotic stricture in ileal than colonic CD well demonstrated the influence of disease location on disease behaviour[6]. As a consequence, the risk of surgery is more important during the natural history of ileal than colonic CD[1]. Currently, the pathophysiology of fibrosis remains unclear and its higher occurrence in ileum than colon is not explained.

In the gut as in other organs, the development of fibrosis is due to an excessive production of extracellular matrix components (ECM) which is at the basis of the obstructive lesion[62]. The ECM is secreted by myofibroblasts deriving from the transdifferentiation of mesenchymal cells (e.g., fibroblasts, smooth muscle cells, stellate cells)[61]. Of note, proliferation and migration of fibroblasts appear as a key event driving intestinal fibrosis[62]. In addition to mesenchymal cells, parenchymal cells can also be a source of myofibroblasts in the context of fibrosis. When injured, IECs can contribute to the fibrotic process by acquiring mesenchymal features and this phenomenon of cellular plasticity is called epithelial-to-mesenchymal transition (EMT)[61]. Intriguingly, we reported evidence (via the measure of 30 markers) supporting the presence of EMT in the ileal ulcer edge of CD patients while this phenomenon was barely detectable in the colon[63]. Thus, in case of lesional process affecting the epithelium, ileum could be more prone to EMT than colon. However, this needs to be demonstrated by functional experiments.

The creeping fat is an expansion of intestinal mesenteric fat (resulting from hyperplasia of adipocytes) which is specifically observed in CD, its presence remains an enigma[64,65]. Interestingly, it has been reported differences between ileal versus colonic mesenteric fat in CD patients: reduced adipocyte size, higher proportion of fibrosed tissue, higher T-cells infiltration and higher level of inflammation[66]. The presence of creeping fat is highly suspected to play a role in fibrosis pathogenesis and location. Indeed, creeping fat develops

and wraps around the intestine primarily in sites of fibrosis and inflammation of the ileum[67]. Thus, this phenomenon forms patches of fat tissues which strikingly follows the behaviour of CD[67]. Given their spatial concomitance, creeping fat and fibrosis are seen as connected pathological processes[64]. The understanding of this relation is limited but some data supports a pro-fibrotic role of creeping fat. In CD patients, the predominant macrophages in creeping fat are the M2-type which are well known to promote fibrosis through their secretion of biomolecules such as transforming growth factor β (TGF-β)[68]. However, the role of creeping fat seems dual, not only harmful, since it could be part of a protective response restricting inflammation and limiting the progression of bacteria. Due to the predominance of M2-type macrophages which highly secrete interleukin 10 (IL-10), creeping fat is viewed as an anti-inflammatory environment[68]. On the other hand, experiments on mice models demonstrated that formation of creeping fat is promoted by the translocation of bacteria from the gastrointestinal tract toward the mesenteric fat[67]. As proposed by authors, the development of creeping fat could be a protective mechanism which prevent the translocation of gut bacteria to the circulation[67]. However, these recent advances do not explain why creeping fat is a characteristic of ileal CD. The understanding of this mystery and its relation with the development of fibrotic stricture are probably necessary steps to find new pharmacological targets[64].

2.4-Higher neutrophil activity in colonic than ileal Crohn's disease

Neutrophils are multifunctional immune cells capable to present antigen, regulate immune response (e.g., Th1 and Th17 differentiation), kill pathogens through phagocytosis, neutrophil extracellular traps and release of lytic granules. In CD, neutrophil infiltration is closely associated with the development of lesions and it constitutes an early histological feature of the disease[13,69]. Given the key role of neutrophils in gut barrier homeostasis[70], some authors

pointed the need to know whether these immune cells act differently depending on their location in the gut[14]. In CD, measure of faecal calprotectin and lactotransferrin, two markers of neutrophils, provided indirect evidence supporting a higher involvement of neutrophils in colonic than ileal lesions. Compared to patients with an active ileal CD, patients with an active colonic CD presented much higher levels of faecal calprotectin (180 vs 1383 µg/g, respectively) and lactotransferrin (10 vs 179 µg/g, respectively)[71]. To explain this result, it has been proposed that lesion surface could be lower in ileal than colonic CD[72,73]. This hypothesis could be true but it needs to be demonstrated since, compared to colon, ileum presents a ~2-fold higher length (3 vs 1.5 m) and an enhanced surface area of around 60–120 times due to the presence of villi and microvilli[74,75]. In a complementary or alternative way, it has been suggested that a degradation of calprotectin along the gastro-intestinal tract could explain difference of its faecal concentration between ileal and colonic CD[73]. However, these proposition are only speculative and they did not consider the possibility that neutrophils could show different activity according to disease location. In a proteomic study, we found evidence well supporting a higher level of neutrophil activity in colonic than ileal ulcer edge of CD patients[63]. When compared to paired control mucosa, ulcer edge mucosa presented a much higher increase of neutrophils markers (including calprotectin and lactotransferrin) in the colon than the ileum[63]. Thus, the measure of neutrophil markers in stools seems not only to reflect the extent and the severity of the affected surface but it could also testify from the localisation of the lesions (ileum versus colon). Given the deleterious role of chronic neutrophil infiltration and their secretory granules (e.g., myeloperoxidase, matrix metallopeptidases) on mucosal wound healing[76–78], our result may signify that the tissue repair process is more impacted by neutrophils in colonic than ileal lesions. At a mechanistic level, the higher microbial load in the large than the small intestine[14] could contribute to explain

why, in the presence of mucosal lesions, neutrophils could be more stimulated in the colon than the ileum.

A difference in neutrophil infiltration between ileal and colonic CD could be also responsible for gut segment-specific immune defences. Indeed, neutrophils are well equipped to communicate and interact with plenty of immune cells. For instance, they can modulate DCs recruitment, T cell differentiation and B cell antibody production[79].

Hence, a better knowledge of the relation between neutrophil activity and disease location could be a basis to develop more individualised therapies for CD patients.

2.5- Faecal calprotectin to monitor disease activity and to predict the risk of relapse: performance according to disease location

In CD, faecal calprotectin is the most recognised biomarker for monitoring the disease activity and the risk of relapse. However, faecal calprotectin seemed less reliable in ileal than colonic CD, a situation that could be linked to different neutrophils activity in these two gut segments (see part 2.4).

It has been reported that, in the case of isolated ileal CD, the concentration of faecal calprotectin did not correlate with the endoscopic (CDEIS), imaging (magnetic resonance enterography) and histologic evaluation of the disease activity[71,73,80]. However, contradictory results were reported and the usefulness of faecal calprotectin to monitor isolated ileal CD remains debated[81,82]. That being said, specific biomarkers for ileal lesions are highly required since access to this gut segment is difficult with endoscopy, it is not systematically performed in clinical routine. This is particularly true in case of inflammatory and fibrotic process affecting the ileo-caecal valve and terminal ileum. The need of biomarkers for ileal lesions is all the more true that in ~75% of the case, CD affects the ileum[1].

In predicting relapse, faecal calprotectin seems to have a lower prognostic value in ileal than colonic CD. In 89 CD patients in clinical remission for at least 6 months and followed during 12 months, the prediction of relapse with faecal calprotectin was improved when patients with isolated ileal disease were excluded (area under the curve, AUC, raised from 0.77 to 0.85)[83]. Similar results were reported in an independent study[84]. In another cohort of CD patients in clinical remission (n=65), it has been found that faecal calprotectin can predict the relapse only in patients with an isolated colonic disease[85]. In IBD patients in clinical remission (n=79), some authors concluded that faecal calprotectin is much more performant to predict the relapse in UC (AUC=0.87) than CD (AUC=0.58)[86]. Given the particularly high proportion of patients with an isolated ileal CD (71%), this study can reinforce the idea that the prognostic capacity of faecal calprotectin is better in colonic than ileal disease[86]. Besides, a review showed that all studies involving UC patients (9 out of 9) reported that faecal calprotectin has a prognostic capacity in predicting relapse while this was not the case for 3 out of 11 studies involving CD patients[87].

Altogether, these data indicate that faecal calprotectin has a diagnostic and prognostic value which vary according to disease location.

2.6-Th1/Th17 profile and disease location

In addition to Th1 cells, Th17 cells are now recognised as key players in CD pathophysiology[88]. In physiological conditions, presence (frequency and absolute number) and response of Th17 cells are higher in the ileum than the colon (see section 1). One study supports this observation in the context of CD. In the ileum but not in the colon of paediatric CD patients, inflamed vs non-inflamed biopsies presented an increase of IL-17A and IL-6 mRNA, i.e., cytokines either produced by Th17 cells or promoting Th17 differentiation, respectively[89]. In this study, IFNγ mRNA was increased in the inflamed biopsies from both

the ileum and colon. Thus, authors concluded that ileal CD could have a mixed profile (Th1/Th17) while colonic CD could have a Th1 profile. More studies are needed to demonstrate this phenomenon in adult cases. At therapeutic level, Th17 response was already targeted. Compared to placebo, the blockade of IL-17A activity by secukinumab or brodalumab in CD patients induced a worsening of symptoms (objectified by the CDEIS) and trials were stopped prematurely[90,91]. This effect seemed not influenced by disease location[90]. At the moment, no evidence supports that targeting Th17 response would preferentially treat the ileal form of CD.

2.7-Efficacy of treatments and disease location

Among the predictors of favourable response to biologics, the effect of disease location remains debated. Some studies found an association between isolated ileal disease and poor response to anti-TNFα while others did not report such finding[92]. As for anti-TNFα, the blockade of integrin α4β7 (vedolizumab) or IL-12/23 (ustekinumab) showed contrasting results regarding an effect of disease location on the response to treatment[92]. However, the situation seems less contradictory when disease activity was evaluated objectively. Endoscopic and histologic evaluation of CD activity demonstrated that maintenance adalimumab (anti-TNFα) induced a better mucosal healing of the distal (rectum, sigmoid-left-transverse colon) than the proximal (right colon and ileum) gastro-intestinal tract[93]. Analogous results were observed with ustekinumab and vedolizumab[94,95]. Thus, current treatments for CD seem to present different efficacy according to disease location. This is probably linked to immunological differences across the gastro-intestinal tract (see section 1). However, the relation between disease location and efficacy of treatment is, to our opinion, not well explored. We deplore that disease location is, in many cases, not evaluated as a potential parameter influencing treatment efficacy. For instance, this situation concerns the

randomised trials evaluating the efficacy of infliximab and adalimumab[96–99]. In addition, current treatments of CD have not been designed to target a specific location of the disease. For instance, TNFα production is not a specific feature of ileum or colon. This situation probably reflects a lack of knowledge regarding the pathophysiological features distinguishing ileal from colonic CD. Given that immune defences present fundamental differences between ileum and colon (see section 1), more precise therapies could be expected in the future.

3-Limits and perspectives

3.1-Relation between NOD2 mutations and α-defensin secretion to explain disease location: history of a controversy

In CD, *NOD2* polymorphisms were the first genetic variants associated with the disease location[7]. CD patients with a *NOD2* mutation have a higher risk to develop an ileal disease[100]. NOD2 is an intracellular receptor recognising the muramyl dipeptide (MDP), a component of bacteria. NOD2 is highly abundant in Paneth cells where it is viewed as a key player for AMPs secretion[50,101]. In a logical manner, some studies investigated whether a defect of AMPs production by Paneth cells could explain the association of *NOD2* mutations with ileal CD[102–104]. In human and mice, *NOD2* deficiency has been associated with a reduced mRNA level of α-defensins in the ileal mucosa[102–104]. However, independent studies were not able to reproduce these results and it led to an intense controversy[105–108]. In the ileal mucosa of CD patients, it has been reported that the reduced mRNA expression of α-defensins is associated with inflammation but not *NOD2* mutations[107]. Such effect has been simply explained by inflammation-induced tissue damage and loss of Paneth cells[107]. In the ileal mucosa of mice, *NOD2* deletion was not associated with a reduced mRNA level of α-defensins[108]. Furthermore, discordant results were unexplained in the studies defending a role

of *NOD2* mutations in α-defensin secretion. Indeed, all the α-defensin studied were not affected by *NOD2* mutations. Compared to wild-type mice, *Nod2*$^{-/-}$ mice infected or not with bacteria did not show a reduction of the α-defensin 5 transcript in crypts[104]. In addition, the mRNA level of α-defensin 6 was not reduced in the inflamed ileum of CD patients carrying *NOD2* mutations compared to their wild-type counterpart[102]. Such results are not well compatible with the hypothesis according to which NOD2 regulates the transcription of α-defensins in Paneth cells.

It is commonly accepted that NOD2 upregulates the transcription of AMPs through activation of the nuclear factor-kappa B (NF-κB) pathway[50]. However, this mechanism has been challenged and, as it was early proposed, the action of NOD2 on AMPs could rather involve post-transcriptional mechanisms[105]. In intestinal epithelial organoids from mouse (mini-gut), MDP or other bacterial components induced neither transcriptional activation of NF-κB nor secretion of lysozyme[109]. In this model, Paneth cells secreted lysozyme upon IFNγ stimulation[109]. In other organoid models (mouse), two studies led to the conclusion that, in Paneth cells, NOD2 regulates lysozyme secretion by promoting its exit from lysosome (via cargo sorting) to dense core vesicles (DCVs)[47,110]. This finding was notably supported by showing that NOD2 deficiency provoked the degradation of lysozyme in lysosome[47]. This research allowed to identify a pathway (NOD2–LRRK2-receptor-interacting serine/threonine-protein kinase 2 (RIPK2)-ras-related protein (RAB2A)) in which NOD2 promotes lysozyme secretion through post-transcriptional mechanisms[47,110]. Remarkably, this pathway was not responsible of α-defensin secretion[47,110]. To explain this result, authors proposed that secretion of each AMP, contained in distinct DCVs, could be regulated by independent signalling pathways[47].

Although attractive, the proposed causal relation between *NOD2* mutations, deficit of α-defensin production and ileal CD is not well supported. In Paneth cells, mechanisms inducing

secretion of AMPs are much more complex than initially thought, the role of NOD2 has been redefined and it is far to be completely elucidated.

3.2-Limitations of genetics to explain disease location

In CD, it has been proposed that disease site is highly influenced by genetics since monozygotic twins and family members are highly concordant (>80%) for this disease phenotype[111]. However, such conclusion is not well supported since twins and family members are *de facto* exposed to similar environmental factors. Actually, genetics alone showed weak capacity to explain disease location. Among the genetic factors incriminated in CD, *NOD2* polymorphisms present the strongest association with disease location (ileal vs colonic disease: OR between 1.82 and 2.50 according to NOD2 variants)[1]. However, their influence on disease location remain weak. Indeed, *NOD2* variants explained only 3.23% of the variance for disease location and the genetic risk score (including known risk loci for IBD) showing the most significant association with disease location classified ileal versus colonic CD with an accuracy of only 57%[1]. In line with these observations, the alpha-diversity of microbiota in stools discriminated ileal from colonic CD while a genetic risk score including SNPs associated with ileal CD (*NOD2* and *ATG16L1*) was not able to explain the disease location[55]. In a general manner, the study of genetic variants is criticised for their weak association with disease phenotype, their weak incorporation into clinical practice and the difficulty of understanding their contribution to pathogenesis[112–114].

The contribution of host genetics to dysbiosis affecting ileal CD have been appreciated by twin studies. The affected twins of pairs (monozygotic or dizygotic) discordant for CD present an identical perturbation of the microbiota than non-twin patients, i.e., an increase of *E. coli*, a depletion of *F. prausnitzii* and a reduction of the bacterial diversity associated with ileal CD[53,56]. Hence, genes seem not determinant factors of the dysbiosis observed in ileal CD.

Although genetics weakly influence disease location, its contribution could vary according to gut segment. It is tempting to propose a higher contribution of genetic variants in ileal than colonic CD since some arguments could support this idea: 1) a predominance of ileal location is classically admitted in the familial forms of CD[115]; 2) six genes have been exclusively associated with ileal CD while only one (*MHC*) has been associated with colonic CD (see introduction); 3) age at diagnosis for isolated colonic CD is higher (~10 years) than for the other sites of CD thus suggesting a weaker influence of genetics[54]. However, this idea could be nuanced since isolated colonic CD showed a higher prevalence in female (65%)[54]. Thus, an unappreciated role of sex-related genes could also contribute to disease location. This intriguing hypothesis needs to be evaluated concomitantly with the potential effect of confounders (e.g., oral contraceptive usage has been associated with isolated colonic CD[54]).

All together, these observations underline that, when considered alone, genetic factors are limited to understand the disease pathophysiology and phenotypes. As others, we point out the need of holistic approaches where genetic and environmental factors are considered as an integrated whole to explain disease location[113].

3.3- Environmental factors and their interactions with genetics to explain disease location

In CD, importance of environmental factors has been notably deducted from its worldwide rising incidence and its significant discordance rate (40-80%) in monozygotic twins[52,116–118]. Environmental factors are probably key determinants of the disease phenotype[119]. More importantly, their interactions with genetic susceptibility brought out complex mechanisms which have shown interesting capacity to influence the disease location.

The link between disease phenotype and host-microbiome interaction has been early demonstrated in the *Il-10*$^{-/-}$ mice model of colitis. When axenic (germ-free), those mice did not develop colitis while it was the case in specific-pathogen-free (SPF) conditions[120]. On the

other hand, antibiotics reduced colitis in *Il-10*$^{-/-}$ mice[121]. Interestingly, bacteria seem not only to trigger inflammation in *Il-10*$^{-/-}$ mice but they also influence its localisation. In germ-free *Il-10*$^{-/-}$ mice, inoculation of different bacterial species (nonpathogenic commensal) induced either proximal (cecum) or distal inflammation of the colon[122]. The kinetic and the severity of the disease was also influenced by the bacterial species inoculated. In germ-free *Il-10*$^{-/-}$ mice transferred to SPF conditions, antibiotics targeting either aerobic or anaerobic bacteria showed regional differences in their capacity to reduce colitis[123]. Analogous results were reported in human. In placebo-controlled trials testing the administration of antibiotics (ciprofloxacin combined with metronidazole or metronidazole alone) in CD patients, the treatments seemed effective (clinical remission) in individuals with disease involving at least the large intestine while it was not the case in patients with disease restricted to the small intestine[124,125].

The link between genetic variants and ileal CD seem to implicate a dysfunction of Paneth cells (see 2.1). However, the study of *ATG16L1* mutations well demonstrated that, taken alone, host genetics is not sufficient to induce Paneth cells abnormalities. Indeed, *Atg16l1* hypomorph (*Atg16l1*^{HM}) mice presented a dysfunction of Paneth cells only when exposed to the murine norovirus[126]. On the other hand, wild-type mice did not develop Paneth cells abnormalities in the presence of norovirus[126]. According to these results, a virus and a genetic predisposition can trigger a specific defect of the ileum but only when present together. Such interaction also needed the presence of bacteria since antibiotics were able to reduce DSS-induced colitis in *Atg16l1*^{HM} mice infected with the norovirus[126].

The link between *ATG16L1* polymorphisms and smoking seems another example showing that disease location results from a combination of environmental exposures and genetic susceptibilities. In general, studies have reported that smoking is more frequently observed in patients with ileal or ileocolonic CD than patients with colonic CD[5,54]. Until recently, it was

totally unknown how smoking could be associated with disease location. It was first observed that the association between IBD and 64 SNPs is affected by smoking behavior[127]. Then, smoking was associated with a higher proportion of abnormal Paneth cells (decreased granules) in CD patients carrying the *ATG16L1*^{T300A} SNP than in CD patients without this mutation[119]. The causal relation between smoking, *ATG16L1*^{T300A} SNP and abnormal Paneth cells was demonstrated in mice and cellular pathways were incriminated (apoptosis, metabolism, TNF-α and peroxisome proliferator-activated receptor-γ)[119]. Other mechanisms could explain the contribution of smoking to the ileal form of CD. In ileal biopsies collected in surgical specimens from CD patients, T-cell receptor (TCR) analysis showed a higher clonal expansion and a reduced TCR repertoire diversity in smokers compared to non-smokers[128]. This phenomenon was associated with a higher risk of postoperative recurrence after ileocolonic resection. Hence, alteration of TCR repertoire could be another mechanism explaining the link between smoking and ileal CD. However, smoking as genetics factors weakly contribute to disease location. Indeed, smoking explained only 1.53% of the variance for disease location[1].

Disease location results from complex relations between host genetics, gut bacteria and environmental factors. Furthermore, as shown with the norovirus (see above), other infectious agents than bacteria could influence the disease location. The contribution of viruses, fungi, phages, archaea and helminths remain underappreciated in the pathophysiology of IBD[52,129]. All these infectious agents could, in interaction with host genetics and environmental factors, favour ileal and/or colonic location of CD. More researches are needed to decipher these complex relations.

4-Conclusion

The ileal and colonic CD are recognised as distinct entities. This consideration is well supported by a combination of clinical (natural history of the disease, efficacy of treatments and monitoring), epidemiological (smoking status, age, gender) and biological (genetics, microbiota, immunology, mesenteric fat) data. However, the pathophysiological mechanisms distinguishing ileal from colonic CD remain poorly understood. New ideas and dedicated works are needed to bridge this gap of knowledge, this should offer opportunities to develop a more individualised management of CD patients.

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Table 1. Main features distinguishing ileal from colonic Crohn's disease

	Ileal Crohn's disease	**Colonic Crohn's disease**
Genetic variants associated with disease location	*NOD2*[7], *LRRK2*[12], *TCF4*[9], *LRP6*[8], *ATG16L1*[10], *KCNN4*[11]	*MHC*[1,130]
Epidemiologic risk factors	Smoking[54]	Female, oral contraceptive usage, older age at diagnostic (~10 years older compared with the other locations)[54]
Natural history	Higher risk for fibrotic stricture[6] and surgery[1]	Higher risk for perianal fistulae[6]
Pathophysiological characteristics	<u>Microbiota alteration:</u> -↓ Diversity[53,55] -↓ Firmicutes phylum (*F. prausnitzii* and *Roseburia*)[53,55] -↑ Proteobacteria phylum (*E. Coli*, AIEC)[54,58,59]	Microbiota close to healthy individuals[53,55]
	Paneth cell dysfunction[131]	
	Presence of creeping fat[66]	Neutrophil activity ++[63]
	Th17/Th1 profile[89]	Th1 profile[89]
Response to biologics (adalimumab, ustekinumab and vedolizumab)	Better mucosal healing in colonic than ileal Crohn's disease[93–95]	

Performance of faecal calprotectin as biomarker	Better performance to predict the relapse in colonic than ileal Crohn's disease[83–85] Better performance to monitor disease activity in colonic than ileal Crohn's disease (controversial)[71,73,80–82]

AIEC: adherent-invasive *E. coli;* ATG16L1: autophagy-related 16-like gene; EMT: epithelial–mesenchymal transition; KCNN4: intermediate conductance calcium-activated potassium channel protein 4; LRP6: low-density lipoprotein receptor-related protein 6; LRRK2: leucine-rich repeat kinase 2; MHC: major histocompatibility complex; NOD2: nucleotide-binding oligomerization domain-containing 2; TCF4: transcription factor 4.

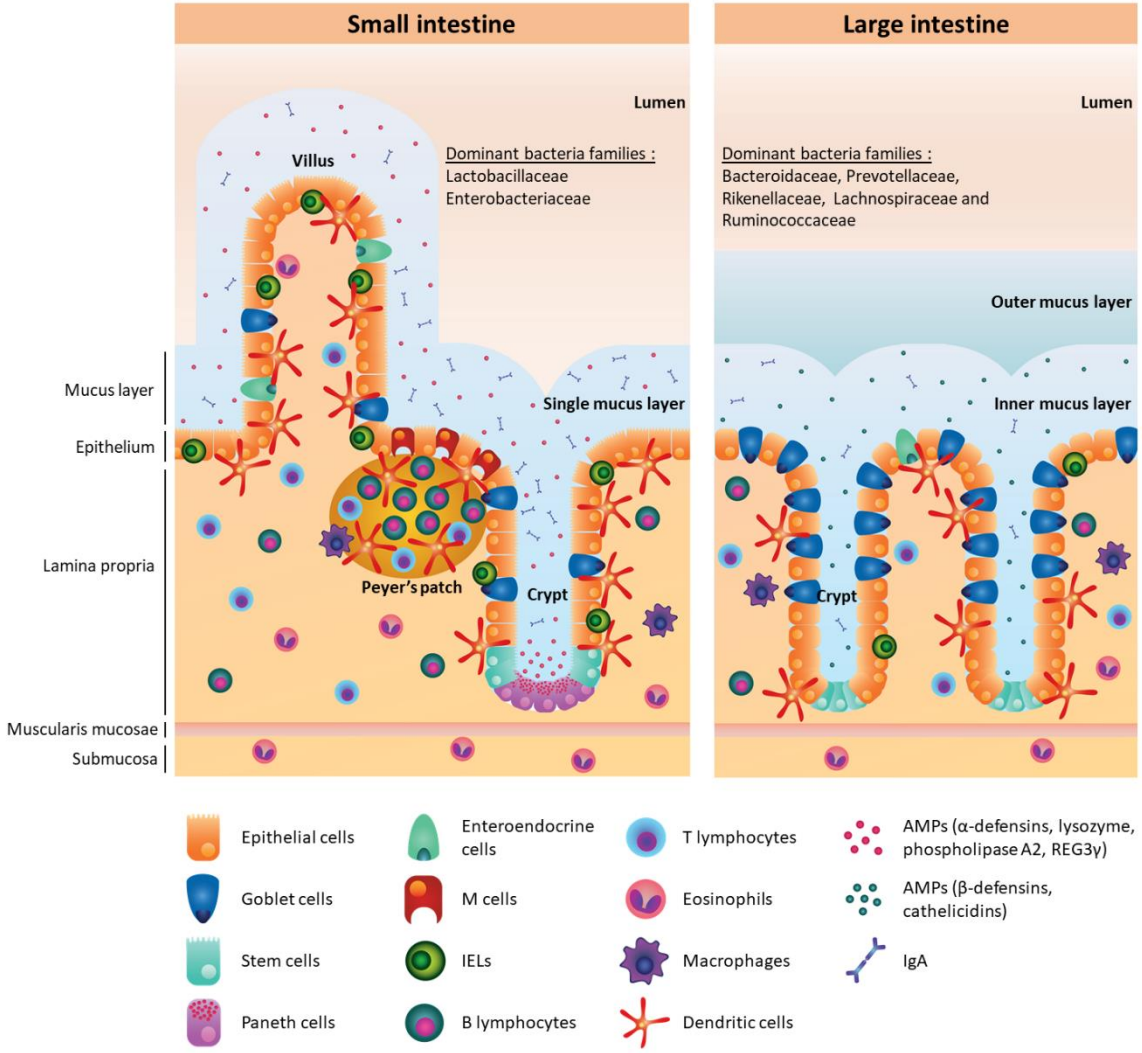

Figure 1. Graphical summary of the physiological features distinguishing the small from the large intestine.

AMPs: anti-microbial peptides; IELs: intraepithelial lymphocytes.