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Crohn's disease : why ileum ?

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Nicolas Richard, Guillaume Savoye, Mathilde Leboutte, Asma Amamou, Subrata Ghosh, Rachel Marion-Letellier

Abstract

Crohn's disease (CD) is an inflammatory bowel disease characterized by immune-mediated flares affecting any region of the intestine alternating with remission periods. In CD, the ileum is frequently affected and about one third of patients presents with a pure ileal type. Moreover, the ileal type of CD presents epidemiological specificities like a younger age of onset and often a strong link with smoking and genetical susceptibility genes. Most of these genes are associated with Paneth cell dysfunction, a cell type located in the intestinal crypts of the ileum. Besides, a Western type diet is associated in epidemiological studies with CD onset and an increasing evidence shows that diet can modulate the composition of bile acids along with the microbiota which in turn affect the ileal susceptibility to inflammation. Thus, the interplay between environmental factors and the histological and anatomical features of the ileum is thought to explain the specific transcriptome profile observed in CD ileitis. Indeed, both immune response and cellular healing processes harbour differences between ileal and non-ileal CD. Taken together, these findings advocate for a dedicated therapeutic approach to managing ileal CD. Currently, interventional pharmacological studies fail to clearly demonstrate distinct response profiles according to the disease location. However, the high rate of stricturing disease in the ileal CD requires the identification of new therapeutic targets to significantly change the natural history of this debilitating disease.

Key Words: Ileum; Crohn disease; Bile acids; Paneth cells; Diet; Genetics

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Core Tip: The ileum is frequently affected by Crohn's disease (CD). Ileal CD differs from other CD types in its epidemiology and natural history. Anatomical and histological features of the ileum provide the keys to understanding this singular

disease. Moreover, we herein discuss the crosstalk that takes place in the ileum between an individual and his environment and the clinical significance.

2 INTRODUCTION

Crohn's disease (CD) is an inflammatory bowel disease (IBD) characterized by repetitive inflammatory flares, and often chronicity. Unlike ulcerative colitis (UC), the other main subtype of IBD, CD can affect any part of the digestive tract. The location of the disease is a critical biological aspect of CD whereas the inflammatory, stricturing or penetrating behaviour is thought to be a reflection of the disease progression[1].

Data on the epidemiology of IBD are provided by population-based studies showing an increasing incidence and prevalence of IBD in the West over the last 50 years[2,3]. In a systematic review pooling every epidemiological study on IBD over the world since 1990, the global prevalence of IBD is higher in western countries (322 per 100000 in Germany) than in newly industrialized countries[4]. In newly industrialized countries like Asia and middle east, epidemiological studies report a rising incidence of CD[4,5]. The Montreal classification distinguish CD involving the ileum, the colon and both the colon and the ileum[6]. About one third of patients with CD presents a disease involvement limited to the ileum and this proportion does not vary between 'Western' and newly industrialized countries[3,5,7]. Once the diagnosis of ileal CD is made, less than one fifth of patients will present colonic lesions over time[3]. In addition, ileal CD occurs in younger patients than colonic CD[1]. These epidemiological observations have led some experts to plead for personalized approaches to therapy based on the disease location.

Even though CD pathogenesis remains elusive, current consensus considers CD a result of genetic, immunological and environmental factors[2]. Of relevance, ileum is the place for the crosstalk of these multiple etiological factors in CD. In this review, we will depict the different physiopathological aspects of the ileal CD and their clinical impact (Figure 1).

Genetic susceptibility

Genetic factors are involved in IBD pathophysiology and genome-wide association studies have linked several genes with the location of the disease. In a large epidemiological study performed in more than 34000 patients with IBD across Europe, North America and Australia, susceptibility genes were determinants of the location of the disease whereas inherited genes showed a loose link with the inflammatory, penetrating or stricturing behaviour of the disease[1]. The genetic variants presented herein sums up the current state-of=the- art but new techniques such as genomic DNA are likely to provide new insight in the next few years.

NOD2

NOD2 is sensor of the innate immune system, able to detect bacterial fragments, specifically muramyl dipeptide[8]. During *in vitro* differentiation of intestinal epithelial cells into Paneth cells, NOD2 signalling can modulate the expression of enteric antimicrobial peptides[9]. Even though intracellular pattern recognition receptor gene NOD2 is widely associated with CD risk[2], mutations in the NOD2 gene are strongly associated with ileal CD and are correlated with a younger age at the diagnosis [1,10,11]. The specific association between NOD2 mutations and ileal CD is partially explained by its distribution along the gastrointestinal tract. Histologically, NOD2 is overexpressed in the ileal crypts compared to colonic crypts[12].

LRRK2

Like NOD2, the leucine - rich repeat kinase 2 (LRRK2) gene is expressed in Paneth cells. LRRK2 gene is implicated in vesicular trafficking, cytoskeleton homeostasis and consequently in inflammation and immune response[13]. More specifically, LRRK2 is overexpressed in Paneth cells and its deficiency causes deprivation of lysozyme in Paneth cells[14]. In a case-control study, mutations of LRRK2 were associated with ileitis and an early onset of CD[15].

MHC

The expression of other genes involved in immune response is likewise implicated in ileal CD. The major histocompatibility complex (MHC) is involved in the presentation of antigen in a large variety of cell types including T-cells. In a recent meta-analyses, several mutations of these genes have been associated with CD especially in the Korean population but also in the European population[16]. Of note, single nucleotide polymorphism of these genes are more common in patients with ileal CD compared with patients with colonic CD[1].

ATG16L1

Among susceptibility genes identified in CD, the allele ATG16L1^{T300A} have been associated with impaired autophagy[17,18]. The expression of ATG16L1 is decreased in CD patients[19]. In 9–10-month-old mice, the specific deletion of *Atg16l1* allele in intestinal epithelium cells (IEC) leads to a transmural CD-like ileitis associated with endoplasmic reticulum (ER) stress[20]. Interestingly, NOD2 mutations are also associated with autophagy defects.

TCF-4

In IBD patients, the reduction of the Wnt-signalling pathway transcription factor Tcf-4 is associated with ileal CD predisposition[21,22]. A reduced expression of the Wnt pathway transcription factor TCF-4 results in a reduced expression of Paneth cell defensin expression[21]. In a murine Tcf-4 knockout model, a decreased expression of alpha-defensin levels and a reduced bacterial killing capacity are observed[21].

KCNN4

Finally, in the array of genes implicated in immune response mediated by Paneth cells, the conductance calcium-activated potassium channel protein (KCNN4) is part of potassium pump in the human intestine[23]. The blockage of this calcium-activated potassium channel protein reduces mouse Paneth cell secretion in response to bacterial

stimulation[24]. In human, mutation of the KCNN4 gene is associated with ileal CD in Australian and New Zealand population[25].

In the susceptibility genes mentioned above, most genes are associated with Paneth Cell dysfunction as illustrated in the Figure 2. We will detail below the potential involvement of Paneth cell in ileal CD location.

The ileum, a specific part of the gastrointestinal tract

Beyond the genetic factors, the histological and anatomical features of the ileum itself partially explain the propensity of this specific part of the gastrointestinal tract to be affected by CD.

Physicochemical environment

The ileum presents a unique chemical microenvironment. The intraluminal pH in the ileum is 7.4, the highest of the human digestive tract as a result of the small bowel mucosal bicarbonate secretion[26]. Comparatively, the intraluminal pH in the caecum is lower, 6.5, due to the bacterial production of short fatty acids by colonic bacteria[26,27]. Digestive tract microbiota functional characteristics are modulated by the pH level. In the environment of the ileum (pH=7.4), short chain fatty acids increase the growth and the motility of a pathobionts whereas, in the colonic environment (pH=6.5), short chain fatty acids downregulate virulence gene expression of these strains[28].

Histological changes in ileal CD

From a clinical point of view, most of histological features encountered in ileal CD are also found in other diseases as backwash ileitis in UC for instance[29]. Albeit epithelioid granuloma are considered as the histological hallmark for the diagnosis of ileal CD, it is not a mandatory prerequisite[30]. In about one quarter of patients with ileal CD, pyloric

gland metaplasia resulting from the expression of mucins genes normally specific to the stomach (MUC5AC and MUC6) can be noted[30,31]. Although numerous other histological features are described in ileal CD, focal crypt irregularities is considered by expert consensus as one of the most reliable sign for CD[30].

From a biological point of view, the most noteworthy change is found in Peyer's patches. Peyer's patches are ileal immune structures characterized by a B-cell germinal center surrounded by a T-cell interfollicular region. These mucosal associated lymphoid tissues can be as "gateways" of the intestine. The epithelium under which the lymphoid follicle is located differs from the surrounding villus epithelium of the ileum. Indeed, the function of this follicle associated epithelium consists in sampling and transporting luminal antigens through M cells and dendritic cells to CD4+ cells[32]. Early histological changes in Peyer's patches are reported in ileal CD such as an increase in mast cells or erosive epithelial lesions [33,34]. Further, the increased number of glial cells in the Peyer's patches of patients with ileal CD results in an enhanced intestinal permeability[34]. Together these phenomena may explain the increased vulnerability of the ileal mucosa to bacterial invasion in CD patients [35].

Enteric nervous system

As mentioned in the previous section, histological changes observed in ileal CD include glial cells and the enteric nervous system (ENS). In patients with ileal CD, both the submucous and the myenteric plexus present an overall increase in the number of neuronal cell bodies, enteroglia and interstitial cells of Cajal associated with an upregulation of apoptosis in enteric neurons and enteric glial cells[36,37]. To that extend, although functional evidence are lacking in the literature to fully support this hypothesis, the increased transit time observed in CD patients could be seen as a consequence of ultrastructural injuries of interstitial cells of Cajal in the myenteric plexus [38,39].

Beyond the role of the ENS on intestinal mobility, the density of enteric glial cells conveys a higher risk of ileal CD recurrence after surgery. Thus, after ileocolonic

resection for CD, the inflammation in or around nerve bundles or enteric ganglia is reported by several clinical studies as a risk factor for CD recurrence[40–43]. In the uninflamed section from ileocolonic samples, the number of S100-positive enteric glial cells was enhanced in patients with a relapsing disease unlike vasoactive intestinal polypeptide or substance P positive cells[44]. Furthermore, the ileum of CD patients harbours a different distribution of enteric glial cells with a higher density of these cells around Peyer's patches. In parallel, the mediators of enteric glial cell increase the permeability of the ileal mucosa in CD patients whereas these decrease the permeability of the mucosa in non-IBD patients[34]. The importance of these findings on the natural history of CD remains to be determined. Especially, the effect of the modulation of the ENS in the neuro-immune interplay needs to be investigated.

Paneth cells

Paneth cells are mostly located in the ileum and nearly absent from the colon[45]. This cellular type is located between intestinal stem cells in the small intestinal crypts. Paneth cells produce not only antimicrobial peptides that regulate host-microbe interplay but also factors such as Wnt ligands modulating the activity of intestinal stem cells. Many of the ileal CD associated mutations discussed before involve cellular pathways of Paneth cells.

Paneth cells are rich in mitochondria to sustain their energy-expending secretory functions. In SAMP1 mice, mice genetically predisposed to CD-like ileitis, the number of Paneth cells is decreased and abnormal Paneth cells are associated with disease progression [46,47]. Likewise, the number of Paneth cells is decreased in the small intestine of CD patients. This observation is made in several ethnic populations and particularly in paediatric cohorts [19,48,49]. Mucosal biopsies from adult CD show ultrastructural abnormalities in mitochondria, especially in CD patients with inflammation (73,3%) but also in inactive CD patients (20.3%) which is not the case for goblet cells and enterocytes[50]. In patients with ileal CD, the count of abnormal Paneth cells correlates with disease activity and is predictive of recurrence after surgery[51].

Some authors hypothesise that this dysfunction in Paneth cells could result from mitochondrial impairment[50–52]. Besides, deletion in Paneth cells in mice of prohibitin 1 (PHB1), a major component protein of the inner mitochondrial membrane implicated in cell respiration, causes ileitis[52]. This effect is mediated by oxidative stress. Furthermore, the use of a specific mitochondrial-targeted antioxidant (Mito-Tempo) ameliorates ileitis in mice . The use of the same mitochondrial-targeted antioxidant (Mito-Tempo) on human ileal biopsies of CD patients normalizes the expression of 25% of altered CD genes among which are genes implicated in antigen processing, lipid metabolism, apoptosis, and IL-17/IL-23 signalling[50].

Immune response

As highlighted by research on immunological processes, Paneth cells are part of a crosstalk with the immune system to maintain intestinal microbial homeostasis and intestinal barrier[53,54]. When biopsies from CD patients are compared according to the inflammation site over the gastrointestinal tract, differences in neutrophil activities are observed. For instance, matrix metalloproteinase-9 (MMP-9) and myeloperoxidase (MPO) are relatively less increased in ulcers edges of ileal CD compared to colonic CD, suggesting less neutrophilic degranulation in ileal CD[55].

Likewise, innate lymphoid cells (ILCs) are mounting in interest as components of the immune system. Three groups are individualized according to their properties: ILC1, ILC2 and ILC3. A switch is noted in the inflamed ileal mucosa of CD patients from a ILC3 phenotype limiting commensal bacteria specific $CD4^+$ T cell response to a ILC1 phenotype associated with IFN-γ production[56]. The aryl hydrocarbon receptor (AhR), a ligand-dependant transcription factor, is involved in this process and downregulated in inflamed mucosa of IBD patients[57,58]. Pharmacological or genetic activation of the AhR enhances ICL3 maintenance conferring a protection against pathogenic bacteria in mice and downregulates ICL2 maintenance implicated in immune response in worm[59]. Agonists of aryl hydrocarbon receptor are provided by the environment, the

commensal flora and the tryptophan metabolism[60]. In patients with ileal CD, impaired tryptophan metabolism is noticed with decreased levels of kynurenine and expression of kynureninase[61].

Concordantly with a typical cellular immune response, ileal CD instigates a specific cytokine profile. Interferon lambda (IFNL) is secreted in response to microbial stimulation or to T-cell–mediated mucosal inflammation. IFNL is upregulated in ileal mucosa of patients with CD and can trigger ileitis in a murine model and induce Paneth cell depletion independently of tumour necrosis factor (TNF)[62]. The effect of IFNL in ileum is mediated by JAK-STAT pathway which represents a promising therapeutic target already being used in the clinic[62].

Besides the role played by IFNL, IL-22 takes part in the pathogenesis of ileal CD. IL-22 is produced by T-cells and ICL3 during IBD flares[63,64]. IL-22 is thought to assume an immunoregulative role as its blockage by the IL-22 binding protein prompts a severe inflammation in a rodent colitis model[64]. In the ileum, interestingly, the levels of IL-22 binding protein are specifically high in comparison with colon[65]. This heterogenous distribution along the gastrointestinal tract may result from the ileal infiltration of eosinophils which product IL-22 binding protein[64,65].

Ileum as endocrine organ

If the ileum encompasses a high proportion of eosinophils cells, an enhanced enteroendocrine cell activity is also found in the ileum[66]. Additionally, terminal ileal chromogranin A cells and GLP-1 positive L-cells are increased in ileal CD specifically[66]. Studies on serum levels of GLP-1 are sparse though and include small cohorts (<20 patients). Data are controversial and show similar serum levels of GLP-1 between ileocolic CD and colonic CD patients but higher serum level of GLP-1 in IBD patients compared to healthy controls[67,68]. Thus, no conclusion can be drawn on the nutritional and immune impact of this discovery.

Besides harbouring enteroendocrine cells, the ileum harbours oestrogen receptor of subtype β whether ileum is affected by CD or not. The expression of the latter subtype is associated with a milder course of the disease in a cohort of 37 patients with ileal CD. Accordingly, inflammation score was inversely correlated with the expression of oestrogen receptor β. Similarly, a higher expression of oestrogen receptor β is found in patients with non-stricturing non-penetrating disease[69]. In a chemical colitis model in rats, activation of oestrogen receptor β reduces inflammation score along with inflammatory pain and inhibits the ionotropic P2X3 receptor[70]. Notwithstanding this observation, although colonic CD is more commonly diagnosed in men, female sex is not independently associated with ileal CD[1,71].

Myofibroblasts

While ileitis is often studied from the perspective of inflammation, little is published about cell repair. The gene encoding tumour progression locus-2 kinase, a proinflammatory enzyme, is associated in genetically invalidated murine model with a surprising homeostatic role, tempering the effect of a chemically induced colitis. Mechanistically, this gene does not directly impact the immune response but plays a critical role in intestinal myofibroblasts which contribute to the healing of intestinal epithelium. In response to inflammatory signals from the microenvironment, intestinal myofibroblasts trigger compensatory epithelial proliferation in the intestinal crypts [72].

Transcriptome studies

The integration of the different pathways mentioned above in the pathogenesis of ileal CD is facilitated by multi-omics studies. Thus, differences in pathogenesis between ileum and colon are highlighted by these techniques. For instance, in CD patients, metabolomic study of non-inflamed ileum and non-inflamed colon biopsies distinguish clearly different profiles. It is noteworthy that these differences blur in inflamed ileum and colon samples[73].

Although physiopathology of ileal CD has been studied as unique phenomenon, emerging evidence advocate for a more heterogeneous process in which pathophysiological pathways differ from one patient to the other. Thus, transcriptomic data analysis from CD ileal tissue sample is able to identify subgroups of patients with distinct recurrence rates after surgery[74].

In a recent transcriptomic study performed on ileal mucosa samples, inflammatory genes (IL-6, IL-8, IL-1B) were upregulated whereas metabolic process genes were downregulated in ileal samples from CD patients compared to controls. Early recurrence of CD after surgery was associated with an overexpression of TNFα, IFNγ, IL23A and IL17A upregulation. In addition, using a regression model to predict CD recurrence after surgery, mitochondrial dysfunction

and JAK/STAT upregulation in the ileum were independently associated with post-operative recurrence[75]. Transcriptomic studies also provide new insight into cell population specificities in ileal CD. When drawing cell type atlas of the ileal mucosa of CD patients, tuft and BEST4+ cells were the cell types associated with CD irrespectively to the treatment status[76]. Tuft intestinal cell is a sparse cell type implicated in the defence against helminthic and protozoan infections[77]. BEST4+ cells represent about 1% of ileal epithelial cells and plausibly contribute to the goblet cells mucus secretion[78]. Surprisingly in this ileal cell types study, immune compartment was slightly affected by CD albeit treatment status in CD patients modify the epithelial and immune compartment[76].

Among the differentially expressed gene in ileal inflammatory response, activating transcription factor 4 (ATF4) is a transcription factor widely expressed in the human body including in the ileum. ATF4 downregulation in intestinal epithelial cells is noted in active CD patients and causes spontaneous enterocolitis in mice while altering ileal Paneth cells' function. Furthermore, murine ATF4 deletion impairs the glutamine uptake of the intestinal epithelium and concordantly glutamine supplementation restores Paneth cells function and decreases intestinal inflammation[79]. A few years

earlier, the inhibition of the ATF4 pathway has demonstrated an altered autophagy in human intestinal epithelial in presence of AIEC. This pathological response results in intracellular bacterial replication of AIEC and consequently to pro-inflammatory patterns[80].

Bile acids

The role of bile acids is largely reported in liver disease in which bile acids are an inflammatory cues and treatment targets[81]. The involvement of the gut in the pathogenesis of inflammatory liver diseases and similarities in the etiological factors led to consideration of the role of bile acids in IBD[82].

Primary bile acids are produced in the liver and secreted through the biliary tree into the gastrointestinal tract. Most primary bile acids are reabsorbed by the ileum and hence recycled several times. A minority of primary bile acids are transformed into secondary bile acids by a narrow range of gut bacteria. The pool of bile acids can influence the composition of gut microbiota which in turn can modulate the bile acid pool composition[83]. Thus, impaired bile acids pools in relation to impaired microbiota enzymatic activities have been described as contributors to the inflammatory loop of IBD[84]. Bile acid composition in the lumen of the ileum differs between CD and non-CD patients with a relative decrease of primary bile acids in the ileum of CD patients[85]. This finding should be taken with caution though as malabsorption of bile acids is documented as a consequence of ileal CD especially in patients with a history of ileal resection[86,87]. Moreover, in a cohort of 166 patients, enhanced primary biliary level in the stool is independently associated with ileitis[88]. In a multi-omics approach based on a stool collection of 200 IBD patients, ileal CD profile is characterized by increased primary and secondary bile acid levels and shifts in taxa in favour of bacteria associated with bile acid-rich environments (*Gammaproteobacteria* and *Blautia sp.*)[89]. Further, secondary bile acids levels in patients with inflammation limited to the ileum tend to increase after biological treatment reaching a similar level with control subjects[90].

In addition, bile acids exert inflammatory modulating properties through the stimulation of FXR. In a murine model of chemically induced inflammation, the activation of FXR demonstrates anti-inflammatory effects through a reduction of epithelial hyperpermeability and of proinflammatory cytokines production[91]. Furthermore, the obstruction of bile flow in mice provokes mucosal ileal injuries reversed by administration of bile acids. In details, this result is explained by the activation of FXR by bile acids which promotes enteroprotective genes and limits ileal bacterial overgrowth[92]. Furthermore, bile acid pool modulation directly affects the ileum and Paneth cells. According to a recent article, Paneth cells number is linked to diet and to microbiota by bile acid in obese CD and non-CD patients irrespectively of other risk alleles (ATG16L1and NOD2).REF ? In fact, in mice fed with a high fat diet, a similar phenomenon is observed and notably, high fat diet alone in germ-free mice as well as microbiome transfer alone in mice fed with standard diet are unable to induce alteration in Paneth cells. The reason of this phenomenon is also dependant FXR activation by bile acid pool. Thus, the conjunction of a high fat diet and *Clostridium*-mediated production of secondary bile acid explains the role of both diet and microbiome in this mechanism[93].

Lastly, bile acids promote the expression by pathobionts of long polar fimbriae which facilitates in the ileum the interplay of these strains with Peyer's patches and bacterial translocations[94]. For example, primary bile acid level is inversely correlated with the abundance of *F. prausnitzii* and its acetate and L-methionine producing enzyme[88]. In patients treated by surgery for ileal CD, bile acid metabolism specificities are associated with ileal recurrence of CD[89].

Nevertheless, there is only limited evidence on the therapeutic role of bile acids in ileal CD. While several authors reported the protective role of ursodeoxycholic acid and of its precursor, the lithocholic acid, against chemically induced colitis in mice[95–97], there is a lack of data about the effect of an oral supplementation with ursodeoxycholic acid in patients with IBD. Thus, only one small single-centre trial examined the effect of ursodeoxycholic acid on UC and none was performed in patients with CD [98].

Microbiota

An increasing number of authors investigate the link between gut microbiota and CD. However, many studies focus on the analysis of DNA extracted from stool. This methodology shed light on the colonic microbiota of the colonic lumen but is unable to draw conclusion on the microbiota associated with the ileum specifically. Moreover, in a systematic review, the study of the microbiota associated with mucosa is regarded as more relevant in the understanding of CD pathogenesis[99]. In addition, dysbiosis is described in some mice model as a by-stander of ileitis. For example, in genetically predisposed mice *Atg16l1*$^{\Delta IEC}$, a dysbiosis is observed but litters cross-fostering predisposes to colitis but not ileitis[20].

Host/microbiota interplay

Microbiota homeostasis plays a critical role into CD physiopathology[2]. Thus, mucosal immunity regulator molecules such as vitamin D receptors have been associated with susceptibility to bacterial and chemical colitis. The genetic inhibition of vitamin D receptors (VDR) in Paneth cells of VDR$^{\Delta PC}$ mice result into lower expression of lysozymes in Paneth cells[19]. VDR inhibition impinge on response to pathogenic bacteria. VDR$^{\Delta PC}$ mice are not only more sensitive to bacterial infection but also to chemical damage. Conversely, this susceptibility in VDR$^{\Delta PC}$ mice is reduced in case of co-housing with non-VDR$^{\Delta PC}$ mice, indirectly suggesting a protective role of the microbiome[19]. In SAMP1/YitFcsJ (SAMP1) mice which develop spontaneous terminal ileitis, dysbiosis occurs during disease progression with a decrease in *Lachnospiraceae* and in *Bacteroides.* In the same animal model, α-defensins misfolding correlates with dysbiosis and could even induce dysbiosis in wild-type mice[47].

In this regard, barrier function of the ileum is also a major feature in the understanding of the host-microbiota interplay. Accordingly, human β-defensin 3 peptide is decreased

and redistributed to the basolateral surface of the ileal epithelium[100]. In parallel, increased enzyme indoleamine 2,3-dioxygenase 1 (IDO1) is found in patients with active CD. This enzyme is the first enzyme in tryptophan metabolism on the kynurenine pathway and is responsible for mucus layer thickening and mucus-associated modulation of microbiota. In a murine enterocolitis model, IDO1 upregulation reduces the abundance of enteropathogenic *E. coli* in the ileum. Likewise, IDO1 downregulates inflammation in response to chemical colitis in mice and augments *A. muciniphila* and *M. schaedleri* abundance [101].

Adherent-invasive *Escherichia coli* (AIEC)

In the nineties, a French team identified a strain of *Escherichia coli,* adherent-invasive *Escherichia coli* (AIEC), which was adherent to the ileum without harbouring virulence factor-encoding genes[102]. AIEC was proved to be associated with ileal CD in further studies and to be able to invade epithelial cells[102,103]. Interestingly, AIEC are able to induce granulomas *in vitro* which is one of the main histologic features of CD[2,104]. The diagnosis of AIEC is classically made on ileal biopsies although a dedicated serology could be also informative and less invasive[105]. The invasive property of AIEC is favoured by the overexpression of the glycoprotein CEACAM6 in the ileal epithelium. This glycoprotein interacts with the bacterial adhesive factor FimH to promote the AIEC-enterocyte interplay in the ileum[106]. Using this receptor, AIEC modulate the ileal epithelium metabolism and induces strong gut inflammation[107]. AIEC induces the expression of hypoxia inducible factor, overexpressed in the ileum of CD patients and promotes barrier defects in the intestinal epithelium paving the way for the onset of inflammation[108].

The relapse of CD in patients after surgical treatment is used to decipher the early steps in CD onset. In a recent prospective, multicentric cohort of patients with ileal resection, AIEC in the remaining ileum was associated with early ileal lesion of a CD recurrence.

The presence of AIEC within the surgical ileal specimen was predictive of the endoscopic recurrence of CD[109].

As highlighted by a recently published article, factors influencing the ileal susceptibility to AIEC are numerous and among them epigenetic regulators also have a modulating activity on a large range of proteins[110]. For this reason, before considering a clinical application in ileal CD, further studies are needed to prevent possible adverse effects induced by the modulation of these epigenetic targets or to identify more specific genes associated with AIEC colonization. Another promising approach would be the use of bacteriophages to target specifically AIEC in ileal disease[111]. Albeit no bacteriophage is approved yet in the EU or in the US for intestinal human therapeutic use, numerous studies reported encouraging results *in vivo* and therefore data about their effect on the human microbiota are needed in the future[111].

F. prausnitzii

In a stool based multi-omics analysis of 200 IBD patients, the abundance of a member of Firmicutes, *Faecalibacterium prausnitzii (F. prausnitzii),* was highly discriminant of ileal CD compared to colonic CD[89]. Even in non-inflamed ileal samples of CD patients, the commensal bacterium *F. prausnitzii* is decreased in comparison with healthy controls which suggests a relevant causal link between this bacteria and the CD onset[112]. Accordingly, a low level of *F. prausnitzii* in the ileal mucosa-associated microbiota is associated with a higher recurrence rate of CD after ileal resection, corroborating the putative role of *F. prausnitzii* in ileal CD pathogenesis[113]. In addition, a recent study confirms the role of *F. prausnitzii* in ileitis after ileocolectomy and points out a possible association with bile salts profile. Thus, elevated levels of primary bile acids levels are associated with a decreased *F. prausnitzii* abundance. These two parameters were the only factors associated with ileitis in this cohort of 166 patients[88]. The administration of *F. prausnitzii* or of its supernatant *in vitro* and *in vivo* counterbalances the gut inflammation by blocking NF-κB activation and IL-8 production[113]. Interestingly, in

CD patients, *F. prausnitzii* correlates specifically with ileitis irrespectively of their genetic background[89,114]. In an original work on twins in whom biopsies were performed along the lower gastrointestinal tract, the abundance of *F. prausnitzii* was specifically decreased in ileal CD, compared to colonic CD or healthy twins[114].

Mycobiota

The study of microbiota in IBD is not limited to bacteria but also includes fungi. Fungal microbiota was strongly suspected to be involved into the CD pathogenesis in particular because of the diagnosis value of anti-*Saccharomyces cerevisiae* antibodies in CD[2]. In a study of 168 ileal biopsies, mycobiota was modified in CD patients with an increased abundance of *Malassezia* and a decreased abundance of *Saccharomyces*. The increase of *Malassezia* was notably associated with a severe evolution of the disease during follow-up[115].

Fungi *Debaryomyces hansenii* is increased in inflamed ileum of CD patients unlike non-inflamed ileum of CD patients. Moreover, oral gavage with *Debaryomyces hansenii* impairs crypt regeneration and wound healing after biopsy injury[116].

Environmental factors

Microbiota bridges the gap between host susceptibility factors and its environment. Most environmental factors are identified by epidemiological studies and associated with the onset of CD or its recurrence after surgery. Few of them though can impede the natural history of CD once the disease established.

Smoking

Cigarette smoking is a well-established risk factor for developing CD[1–3]. In patients with CD, the relative statistical weight of smoking was bigger than the relative weight of the genetic variants presented in the previous section[1]. Besides, cannabis is frequently used as a symptomatic treatment by patients with CD involving ileum and associated with tobacco[117]. Regarding cannabis use, in a double-blind, randomized, placebo-controlled, cannabis oil induces clinical improvement without any endoscopic change[118].

Regarding tobacco, in mice cigarette smoke extract induces intestinal inflammation and morphometric changes in ileal epithelium regardless of the way of administration (intragastric or intraperitoneal), advocating for a systemic effect[119]. Moreover, mice exposed to cigarette smoking are more likely to develop pathological inflammation in response to bacterial inflammation which could be the hallmark of an impaired expression of antimicrobial peptides[119]. Besides, smoking is associated with a reduced number of normal Paneth cells in the ileum in patients presenting a CD susceptibility allele ($ATG16L1^{T300A}$) as well as in mice presenting this genetical susceptibility. This defect in the ileum is mediated by Paneth cells apoptosis driven by the activation of peroxisome proliferator-activated receptor gamma (PPARγ) and prevented in mice by the use of anti-TNFα drugs[120].

Moreover, smoking is associated with an upregulation of angiogenesis in smokers with CD compared to their non-smoking counterparts. Importantly, mice exposed during 8 weeks to cigarette smoke present mucosal tissue hypoxia associated with an increased expression of pro-inflammatory cytokines and of angiogenic factors whereas smoking cessation reverses this process in the ileal mucosa. In addition, the exposure to smoke drives an increased sensibility to chemically induced colitis[121]. Although genes of hypoxia-inducible factor are overexpressed in the inflamed ileum and in the adjacent mesenteric tissue of CD patients, hypoxia in mice do not impact experimental ileitis[122–124]. All together, these results advocate for a specific impact of tobacco smoking in ileitis pathogenesis.

Evidence for other environmental factor is sparce in the literature. Recently, serum levels of bisphenol A, a component used in manufacture of various plastics, have been linked to the inflammatory status in CD patients. Furthermore, patients with bacterial DNA translocation present higher serum level of bisphenol A associated with a reduced expression of tight junction genes[125].

Diet

Western diet is characterized by low intake of fibres contrasting with high intake of refined sugar, animal protein and total fat. Numerous links between diet and IBD are established[126]. To that extent, diet modulates microbiota associated with ileal epithelium. Thus, adherent-Invasive *Escherichia coli* (AIEC) are associated with hyperacetylated histone H3 ileal epithelium and consequently histone deacetylase enzymes control the entry of AIEC in intestinal epithelium cells and in a murine model. Interestingly, a high fat diet enhances the histone acetylation in mice compared to a standard diet[110]. Overweight (body mass index >25kg/m^2) in IBD and in non-IBD patients is associated with Paneth cells defects in ileal epithelium. This defect can be induced by Western diet in mice[93].

Regarding fat intake, the ratio between n-3 and n-6 polyunsaturated fatty acids (PUFAs) is unbalanced in favour of an excessive intake of n-6 PUFAs in Western diet. Oral feeding with n-3 PUFAs in SAMP1/Yit mice ameliorates histological features of ileitis, decreases addressin molecules expression (MAdCAM-1) and consistently lymphocytes infiltration in the ileum[127]. To that extent, oral supplementation with linseed oil rich in α-linolenic acid (n-3 PUFAs) in a murine model with physical activity reduced inflammation after oral challenge with AIEC[128]. However, oral n-3 PUFAs supplementation has never shown definitive results in patients with CD[129]. In all likelihood, n-3 PUFAs alone are unable to stop the inflammatory loop once started but remain putative candidates to prevent the onset of ileal CD.

Fibre intake is identified as a protective factor against CD onset but not UC[130]. Although such epidemiological data are based on the study of a 40-year-old population, fibre intake and more accurately inulin supplementation can modulate PPARγ signalling pathway in pigs[131].

Vitamin D deficiency has been reported in IBD patients by numerous studies reviewed elsewhere as an explanation of the North-South gradient of IBD prevalence[132]. In mice fed with vitamin D deficient diet, miR-142-3p expression is upregulated, culminating in a reduction of ATG16L1 and autophagy specifically in ileal Paneth cells. In a paediatric cohort of IBD patients, colonic samples display likewise an enhanced expression of miR-142-3p statistically associated with low serum vitamin D levels[133]. As discussed previously, VDR is overexpressed in the ileum and involved in adequate response to enteropathogens[19]. When associated with a high fat diet, vitamin D deficiency as well as genetic VDR inhibition prompts deficiency in Paneth cell defensins secretion and gut permeability conducing to endotoxemia and systemic inflammation[134].

Last, Western diet is also characterized by high intake of dietary additive related to the consumption of ultra-processed food. To that extend, dietary emulsifiers carboxymethylcellulose and polysorbate-80 are associated with an increased virulence and enrichment of ileal pathobionts[135].

More generally speaking, diet in case of ileal CD should fit the recommendation led by the European Society of Nutrition. The findings discussed above and other have led to counsel a balanced diet rich in fruit and vegetables and rich in n-3 fatty acids. Consequently restrictive diet shall be avoided to avoid risk of deficiencies[136]. As highlighted by this European consensus, good quality data regarding the effects of experimental diets are lacking in the literature. Especially, randomized control trials are sparse to recommend a more specific diet in patients with active ileal CD[136].

Clinical implications

The factors summarised in the previous paragraphs give rise to a distinctive natural history of the ileal CD with specific clinical consequences and interventions necessary.

Natural history of ileal CD

In CD, the time from diagnosis to the first surgery is the shortest in ileal CD compared to the other disease locations. Thus, median time to surgery in ileal CD is about 6 years. Thirty years after the diagnostic of ileal CD, almost every patient with ileal CD will have undergone at least one surgery according to a broad international study[1]. At 12 months after surgery, recurrence at colonoscopy is observed at and above this anastomosis in 73% of patients in absence of any treatment[137]. These data regarding recurrence rate published 30 years ago led to development of preventive strategies after surgery.

In terms of behaviour, ileal CD is more likely than colonic CD to be complicated be penetrating or stricturing lesions[3]. These severe manifestations contribute to the high rate of surgery. Accordingly, strictures are challenging complications that are usually unresponsive to medical therapy in the absence of surgery[3]. Strictures result from the accumulation of fibrotic protein in the extracellular matrix produced by fibroblasts which partially derived from epithelial cells *via* epithelial–mesenchymal transition (EMT)[138]. Accordingly, based on the comparative study of ileal *versus* colonic ulcers, EMT appears to be a highly noticeable feature in ileal ulcers of CD on the contrary of colonic ulcers of CD[55]. Ileal strictures are likely to present mesenteric fat wrapped around the stricture, known as creeping fat[139]. Creeping fat is a rising interest feature, rich in T-cells which harbours viable bacterial translocation and leads to a pro-fibrosis M2-type microenvironment[140–143]. Remarkably, creeping fat associated with the ileum presents a 10 times higher concentration in T-cells than the colonic fat[144]. In addition, adipocyte hyperplasia is observed in ileal fat unlike in colonic fat[144].

Beyond the ileum

The presence of ileal crypt in colon of CD patients had already been reported previously[12,45]. Recently, the presence of ectopic ileal crypt of the colon have been used to discriminate the IBD subtype in patients with indeterminate (unclassified) colitis. The presence of ileal metaplasia in the colon was strongly associated with a final diagnosis of colonic CD [145]. This interesting discovery could give to the understanding of ileal physiopathology a pivotal role in the understanding of Crohn's disease.

In a specific statistical model based on HLA types and single nucleotide polymorphism, colonic CD appears to be an intermediate between UC and ileal CD[1]. Consecutively, the knowledge of the ileal CD may pave the way to the understanding of other sub-phenotypes of CD.

A dedicated treatment for ileal CD?

Logically, the singularity of the ileal CD mentioned in this review should lead to a dedicated treatment. Nevertheless, ECCO current guidelines on medical management of CD do not advocate for a specific treatment in case of ileal CD[146]. Indeed, studies are controversial. Some studies report a lower rate of response to infliximab, an anti-TNF treatment[147,148]. Nevertheless, numerous studies do not describe such a difference in response rates with anti-TNF[149]. This discrepancy may result from bias such as the extent of the disease irrespectively to the location. Interventional trials specifically dedicated to the study of the ileal CD treatment are henceforth required. In the literature, colonic CD is more likely to respond to treatment compared to ileocolonic CD and mucosal healing may be more difficult to acieve in ileal disease. Likewise, ileal stricturing CD may have lower response rates[149].

As ileal stricture may develop despite of the currently available CD treatment, an American team sought to determine the gene pattern associated with an ileal stricturing CD in a paediatric cohort. This analysis identified a long-chain fatty acid, the eicosatetranoic acid a possible antifibrotic tool[150]. To confirm this hypothesis, the same team exposed human intestinal organoids to eicosatetranoic acid reduces the fibrosis

and improves stiffness[151]. In parallel butyrate, a short-chain fatty acid, also downregulated fibrosis according to the same protocol[151].

CONCLUSION

The histological and anatomical features of the ileum are direct answers to the question "why ileum?". Furthermore, the recent works presented in this review highlights the specific interplay between environmental factors like smoking or diet and the ileum. Currently, the prevailing need for surgery in a significant proportion of ileal CD testifies to the urgent need of dedicated pharmacological approaches. As the ileum is the place of the interplay between host characteristics and environmental influences, the deciphering of molecular underpinnings of this dialog is crucial to unveiling new therapeutic targets.

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