

Interleukin-10 and Interleukin-10–Receptor Defects in Inflammatory Bowel Disease

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Abstract Inflammatory bowel disease (IBD) is a chronic inflammatory disease characterized by abdominal pain, bloody diarrhoea, and malabsorption leading to weight loss. It is considered the result of inadequate control of an excessive reaction of the immune system to the resident flora of the gut. Like other primary immunodeficiencies, IL-10 and IL-10 receptor (IL10R) deficiency present with IBD and demonstrate the sensitivity of the intestine to any changes of the immune system. Both IL-10 and IL10R deficiency cause severe early-onset enterocolitis and can be successfully treated by hematopoietic stem cell transplantation (HSCT).

Keywords Interleukin-10 · IL-10 · IL-10 receptor · Mutation · STAT3 · Inflammatory bowel disease · Colitis

Introduction

Inflammatory bowel disease (IBD), in the traditional sense has been divided into 3 conditions: Crohn disease (CD), ulcerative colitis (UC), and IBD unclassified/indeterminate colitis (IBDU/IC). They typically present in adolescence or adulthood, follow a chronic relapsing course, and commonly

share symptoms of chronic abdominal pain, weight loss, diarrhoea, and blood loss per rectum [1, 2].

Several explanations of possible pathomechanisms in IBD have been published with an inadequate immune response to the resident flora of the gut as one of the most popular ones [3–6]. Genome-wide linkage and association studies identified a number of genes that may render individuals more susceptible to IBD [7, 8], but IBD can also result from monogenic defects as demonstrated in primary immunodeficiencies including Wiskott-Aldrich syndrome, nuclear factor κ B essential modulator (NEMO) deficiency, chronic granulomatous disease, X-linked inhibitor of apoptosis (XIAP) deficiency, and IPEX (immunodysregulation-polyendocrinopathy-enteropathy-X-linked) syndrome [9–14].

IL-10 and IL-10 receptor (IL10R) deficiencies add to the group of primary immunodeficiencies that result in severe dysregulation of the immune system, in particular of the intestine [15, 16]. Affected patients usually come down early in life with an IBD-like phenotype accompanied with severe perianal disease, but they may show other clinical features including chronic folliculitis, recurrent respiratory diseases, and arthritis [15, 16, 17, 18, 19]. Abscess formation, anal fissures, and enterocutaneous or recto-vaginal fistulae complicate the disease and frequently require partial or total colectomy. IL-10/IL10R deficiency is typically refractory to immunosuppressive therapies including steroids, methotrexate, thalidomide and even anti-TNF α monoclonal antibodies [15, 16, 17, 18, 19].

IL-10 and IL10R deficiencies are rare, life-threatening diseases, have poor long-term prospects, and are difficult to control with conventional immunosuppressive therapies. Thus, hematopoietic stem cell transplantation (HSCT) has

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been referred to and proved a curative therapeutic approach that induced remission in several patients [15•, 18•].

In this review, we discuss the clinical phenotypes in IL-10/IL10R deficiencies, standard diagnostic procedures, and treatment options.

Clinical Presentation of IL-10/IL10R Deficiency, Differential Diagnoses, and Treatment Options

Only few reports on IL-10/IL10R deficiency have been published so far [15•, 16•, 17•, 18•, 19•, 20•]. Patients with IL-10/IL10R deficiencies typically come down within the first year of life with intractable therapy resistant IBD and severe perianal disease. Even though IBD is at the center of attention, the affected patients may present with other symptoms including folliculitis, arthritis, and respiratory diseases [15•, 16•, 17•, 18•, 19•]. Despite completely abolished IL-10-signaling, the immunological work-up shows only slight abnormalities. Patients may reveal hyper-gammaglobulinemia due to the on-going inflammation, but may also show reduced serum levels of immunoglobulins, which might be the reason for an increased susceptibility to infections [15•, 18•].

Glocker et al were first to describe 4 children with homozygous mutations in the genes encoding *IL10R*, leading to abolished IL-10 signaling [15•]. Two children harboured homozygous missense mutations in *IL10RA* (the gene encoding IL10R2, the beta-unit of IL10R), 2 other had mutations in *IL10RB* (the gene encoding IL10R2, the beta-unit of IL10R). The patients were born to consanguineous parents of Lebanese and Turkish origin and presented within the first 3 months of life. Histology revealed infiltration of the epithelium with inflammatory cells and showed deep ulceration of the intestinal mucosa and abscess formation. Conventional immunosuppressive therapy including corticosteroids and anti-TNF α monoclonal antibodies did not induce remission, and patients underwent multiple surgical interventions including sub-total colectomies. The same group identified 2 other unrelated patients with severe colitis which resembled IL10R deficiency [16•]. Both patients harbored homozygous point mutations in IL-10 (Gly113Arg). In contrast to wild-type IL-10, this mutant IL-10 failed to induce STAT3 phosphorylation and to inhibit LPS-mediated TNF α release in peripheral blood mononuclear cells [16•].

After this initial description of IL10R deficiency other reports on this intriguing primary immunodeficiency have been published. In a cohort of 13 pediatric IBD patients with early-onset, Begue et al identified 2 boys with a mutant IL10R: 1 patient with a homozygous mutation in IL10R1 (Arg262Cys) and another one with a homozygous mutation in IL10R2 (Glu141Stop) [17•]; Moran et al reported a

Canadian girl with a mutation in IL10R1 (Pro206X) [19•], and Mao et al published a report on a boy with a compound heterozygous mutation in IL10R1 (Thr84Thr/Ile; IL10R1 Arg101Arg/Trp) [20•]. In a most recent publication, Kotlarz et al presented 12 more patients with mutations in IL-10 or IL10R (Table 1) [18•]. The majority of patients were born to consanguineous parents and are of Arabic or Pakistani origin, and revealed additional symptoms including chronic folliculitis and arthritis [18•].

IL-10/IL10R deficiencies are not the only monogenic diseases that present with IBD. Wiskott-Aldrich syndrome, NEMO deficiency, XIAP deficiency, chronic granulomatous disease (CGD), and IPEX syndrome may be associated with IBD, and need to be considered in patients in whom IL-10/IL10R mutations have been ruled out [9–14]. Most recently, ADAM17 deficiency has been identified as another monogenic disorder associated with milder IBD and more severe inflammatory skin disease [21•].

Clinicians need to bear in mind that IL-10 and IL10R deficiencies are rare; patients may present with similar severe clinical phenotypes but wild type IL-10/IL10R [22]. The identification of a mutation in IL-10 or IL10R, however, justifies HSCT. The clinical course of IL-10/IL10R deficiencies is life-threatening and without advanced medical support most likely fatal; thus HSCT should be considered as a potential therapy option from early on. The benefit of HSCT has been demonstrated in other primary immunodeficiencies as well: it is the treatment of choice in patients with severe combined immunodeficiency (SCID) and a wealth of experience has been gained since the first successful transplantation from a HLA-identical sibling donor in 1968 [23]. Today, the overall survival for patients with SCID at 1 year has reached 90 % [24]. HSCT with well-matched donors was, so far, carried out in 7 patients with IL-10/IL10R, induced remission, and restored IL-10 signaling [16•, 18•].

What Do We Need IL-10 For?

Our gut is a sensitive ecosystem that is colonized by a plethora of different bacterial species and continuously challenged by food antigens, which results in permanently ongoing pro-inflammatory events. The intestinal integrity is maintained by several factors including the epithelial cell layer, mucus-secreting goblet cells, antimicrobial peptides-producing Paneth cells and Peyer's patches, but IL-10 appears to be critical to keep pro-inflammatory activities in check, to prevent excessive inflammation and to avert tissue damage [25]. The role of IL-10 as a key anti-inflammatory cytokine has been known for years and was first demonstrated in murine models: mice lacking functional IL-10 (*IL-10*^{−/−}) or the IL10R2 subunit (*IL10Rb*^{−/−}) developed severe

Table 1 Gender, ethnicity, and kind of mutation in *IL-10*, *IL10RA*, and *IL10RB* published thus far

Patient	Gender	Ethnicity	Mutation	Reference
1	Male	Turkish	IL10R2: Trp159X	Glocker et al 2009 [15•]
2	Female	Turkish	IL10R2: Trp159X	Glocker et al 2009 [15•]
3	Female	Arabic	IL10R1: Gly141Arg	Glocker et al 2009 [15•]
4	Male	Caucasian	IL10R1: Thr84Ile	Glocker et al 2009 [15•]
5	Female	Pakistani	IL10: Gly113Arg	Glocker et al 2010 [16•]
6	Male	Pakistani	IL10: Gly113Arg	Glocker et al 2010 [16•]
7	Male	not reported	IL10R1: Arg262Cys	Begue et al 2011 [17•]
8	Male	not reported	IL10R2: Glu141X	Begue et al 2011 [17•]
9	Male	Turkish	IL10R1: Arg101Trp	Kotlarz et al 2012 [18•]
10	Male	Turkish	IL10R2: Trp159X	Kotlarz et al 2012 [18•]
11	Male	Latin American	IL10R1 Tyr57Tyr/Cys; IL10R1 Arg117Arg/Cys	Kotlarz et al 2012 [18•]
12	Female	Asian	IL10R2: Cys66Tyr	Kotlarz et al 2012 [18•]
13	Male	Arabic	<i>IL10RB</i> : 3' UTR: c.*C52T	Kotlarz et al 2012 [18•]
14	Female	Caucasian	IL10R2: Trp204Trp/X; IL10R2 Ser230Ser/X	Kotlarz et al 2012 [18•]
15	Female	Caucasian	IL10R2: Trp204X	Kotlarz et al 2012 [18•]
16	Male	Black	IL10R1: Ile169Thr	Kotlarz et al 2012 [18•]
17	Male	Arabic	IL10: Gly153Asp	Kotlarz et al 2012 [18•]
18	Male	Arabic	IL10: Gly153Asp	Kotlarz et al 2012 [18•]
19	Male	Arabic	IL10: Gly153Asp	Kotlarz et al 2012 [18•]
20	Male	Turkish	<i>IL10RB</i> : c.331+907_574del	Kotlarz et al 2012 [18•]
21	Female	Caucasian	IL10R1: Pro206X	Moran et al 2012 [19•]
22	Male	Asian	IL10R1 Thr84Thr/Ile; IL10R1 Arg101Arg/Trp	Mao et al. 2012 [20•]

enterocolitis [26–28]. Mice that lack IL-10 producing regulatory T (Treg) cells due to mutations in the cell lineage defining transcription factor forkhead box P3 (FoxP3) come down with multiorgan inflammation and autoimmune enteropathy [29, 30]. Humans with mutations in the FOXP3 gene present with a similar phenotype and suffer from the IPEX-syndrome, which is characterized by a lack of CD4⁺/CD25⁺/FoxP3⁺ Treg cells. Similar to mice, affected individuals develop autoimmune lymphoproliferation and multiple autoimmune disorders [31].

Beyond the cohort of patients with aggressive early-onset IBD and functionally proven IL-10 pathway defects due to IL-10/IL10R mutations, genome-wide association studies also suggested an important role of IL-10 in the pathogenesis of adult IBD. Franke et al reported the single nucleotide polymorphism (SNP) rs3024505, which flanks the IL-10 gene to be significantly associated with UC in adult patients [32]. A genome-wide association study in a pediatric population by a Canadian group found a significant allelic association between two further IL-10 SNPs (rs2222202 and rs1800871) and CD [33].

Noguchi et al investigated the CD-associated frameshift insertion 3020insC in the intracellular sensor molecule nucleotide-binding oligomerization domain containing 2 (NOD2). They found that the mutant NOD2 actively

inhibited IL-10 transcription. The mutation resulted in an impaired phosphorylation of the ribonucleoprotein hnRNP A and reduced binding to the IL-10 locus, which might explain the intestinal hyper-inflammation in CD [34].

Patients with severe early-onset IBD and loss-of-functions mutations in IL-10 or IL10R demonstrate the necessity of IL-10 for the integrity of the immune system and support the hypothesis that the intestine is particularly delicate to changes of the immunological balance.

Impact of IL-10/IL10R Mutations on IL22, IL26, and IFN λ Signaling

In contrast to IL10R1, which is unique to IL10R and exclusively expressed on hematopoietic cells and tissues, IL10R2 is shared by the IL22-, IL26-, and the IFN λ -receptors (IL28A/B and IL29) and expressed on a wide range of non-immune cells including epithelial cells, and keratinocytes, suggesting that IL10R2 mutations might be associated with a more severe phenotype [35–37].

The chronic folliculitis observed in some of the IL10R2-deficient patients might at least in part be due to hampered IL22 signaling. IL22 has been shown to control immunity of the skin by upregulating the expression of the β -defensins 2

and 3 and the antimicrobial heterodimer S100A8/9 [38, 39]. In mice, IL22 was shown to protect against colitis and to significantly improve colitis [40]; it upregulates expression of antimicrobial proteins and increases mucus production, thereby maintaining the epithelial barrier and protecting from infections [41, 42]. Thus, loss of IL22 signaling might have dramatic consequences and aggravate colitis in IL10R2-deficient patients.

In murine lung epithelial cells, IL22 regulates the innate immune defence by mediating expression of lipocalin-2, a protein capable of sequestering iron from gram-negative bacteria and improving the killing of *Klebsiella pneumoniae* [43]; this might be of relevance in the protection against respiratory infectious diseases.

Even though the λ -interferons IL28A, IL28B, and IL29 were shown to be involved in antiviral immunity [44, 45], they might be of minor relevance since other critical antiviral defences such as the IFN type I and II signaling pathways are still intact. Further studies, however, are needed to rate their importance.

IL-10: Effective as a Drug in IBD?

IL-10 is a multifunctional cytokine, which targets T cells, monocytes, macrophages, and most other hematopoietic cell types [46]. It has, therefore, been of great interest to replicate its immune-regulatory potential for IBD therapy. First hopes were supported by *in vitro* studies confirming the down-regulation of pro-inflammatory cytokine release after exposure to exogenous IL-10 [47]. *In vivo* studies followed with unfortunately less encouraging results: parenteral application of IL-10 to therapy refractory patients with CD did result in some clinical improvement but no significant differences in the induction of clinical remission [48]. In a 24-week double-blind placebo-controlled study of 95 patients with moderately active CD only 23.5 % of patients receiving 5 mcg/kg of human recombinant IL-10 experienced clinical remission and endoscopic improvement compared with 0 % of patients receiving placebo. Higher doses were less effective with the same side effect profile [49]. Two factors most likely contributed to the lack of success: IL-10 has a pro-inflammatory effect by upregulating MHC II expression on B lymphocytes and can induce cytotoxic T-cell differentiation. It has also been shown that at higher doses IL-10 upregulates proinflammatory cytokines such as IFN- γ [50]. Furthermore, IL-10 has a short serum half-life of less than 3 hours, which might be not sufficient to consistently reach target tissue [51].

Targeted enteral application of IL-10 for the treatment of IBD has therefore been of great interest. Steidler et al genetically engineered the non-pathogenic bacterium *Lactococcus lactis* (*L. lactis*) to produce IL-10 and could prove a 50 % reduction of colitis in a dextran sulphate sodium (DSS) murine model. The application of genetically

engineered *L. lactis* to *IL-10*^{-/-} mice led to prevention of onset of colitis [52]. In the first clinical study, 10 patients were included in a placebo-uncontrolled phase I trial with transgenic bacteria *L. lactis* (LL-Thy12) in which the thymidylate synthase gene was replaced with a synthetic sequence encoding mature human IL-10. Treatment was safe and in addition, a decrease of disease activity was observed [53].

In a different treatment approach Nakase et al used IL-10 containing gelatine microspheres (GM-IL-10) and observed macroscopic and histological improvement in *IL-10*^{-/-} mice after rectal application [54]. Expression of IL-12 mRNA and CD40 in Mac-1-positive cells post GM-IL-10 was decreased compared with systemic IL-10 treatment alone.

Gene therapy has been established with variable success *in vitro* and in murine models. Adenoviral vectors encoding IL-10 administered rectally were applied successfully in the murine *IL-10*^{-/-} colitis model by Lindsey et al [55]. Intraperitoneal injection in a rat model was successful in preventing but not in reversing dinitrobenzene sulphonic acid induced colitis in the rat [56]. Bahavsar et al successfully established oral gene therapy in a murine model with a nanoparticles-in-microsphere oral system (NiMOS), which was formulated with murine IL-10-expressing plasmid DNA [57].

Van Montfrans et al engineered primary murine CD4⁺ cells to express IL-10. These retroviral vector transduced cells were able to prevent colitis induced by CD45RB (high) CD4 (+) cells transferred to severe combined immunodeficient mice, but failed to offer therapeutic benefit in acute trinitrobenzene sulfonic acid (TNBS)-induced colitis [58].

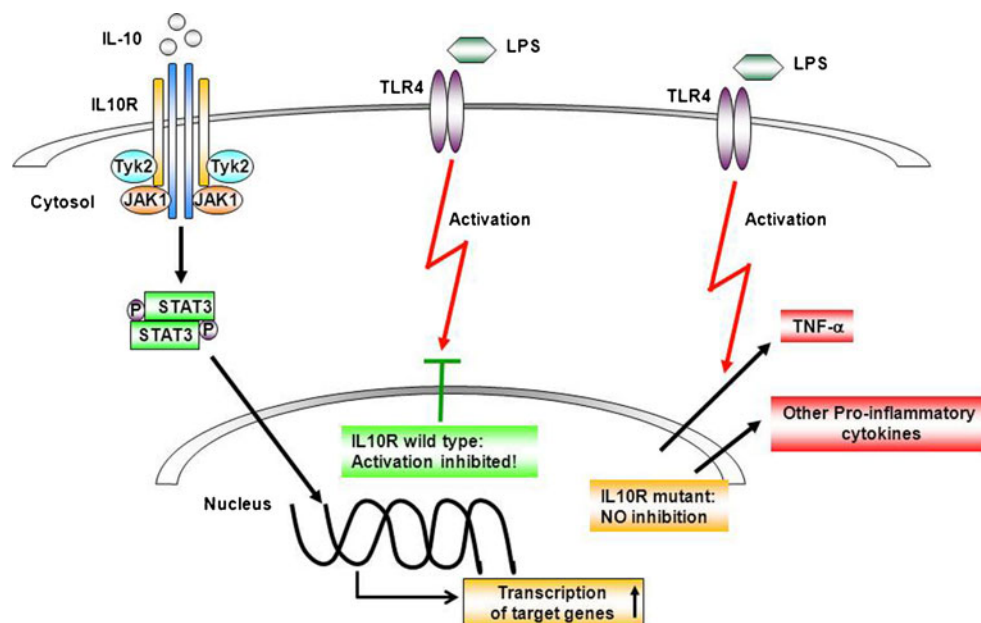
In a murine model, human umbilical cord blood cells were administered to *IL-10*^{-/-} mice without immunosuppression. There was no evidence of graft vs host reaction and the transplanted cells were capable of producing IL-10 over the entire study period of 5.5 months [59]. Bamba et al could demonstrate ameliorated disease activity after bone marrow transplantation of *IL-10*^{-/-} mice with wild type bone marrow [60].

Taken together, the experience with IL-10 as a therapeutic intervention is rather daunting; due to the resistance to standard immunosuppressives and in line with ours and other's results, HSCT poses the best therapeutic approach to cure patients suffering from IL-10/IL10R deficiency and should be aimed for as early as possible [15•, 16•, 18•].

IL-10 Signaling Pathway and Diagnosis of IL-10 and IL10R Deficiency

IL10R is a tetrameric cell-bound structure made up of 2 molecules of the IL10R α -chain (IL10R1) and 2 molecules of the accessory IL10R β -chain (IL10R2) [33, 44, 61, 62]. Binding of IL-10 dimers to the receptor gives rise to the activation of Janus tyrosine kinases (JAK)1 and Tyk2, which activate each other and then phosphorylate IL10R1, thereby

Fig. 1 IL-10 signaling pathway. IL-10 signals via the JAK/STAT3 signaling pathways, which results in phosphorylation of STAT3. Phosphorylated STAT3 dimerizes, translocates to the nucleus, and enhances the transcription of its target genes. In PBMC from IL-10R wild type individuals, IL-10 inhibits LPS-mediated release of pro-inflammatory cytokines including TNF α . In IL-10R mutant individuals, IL-10 has no effect



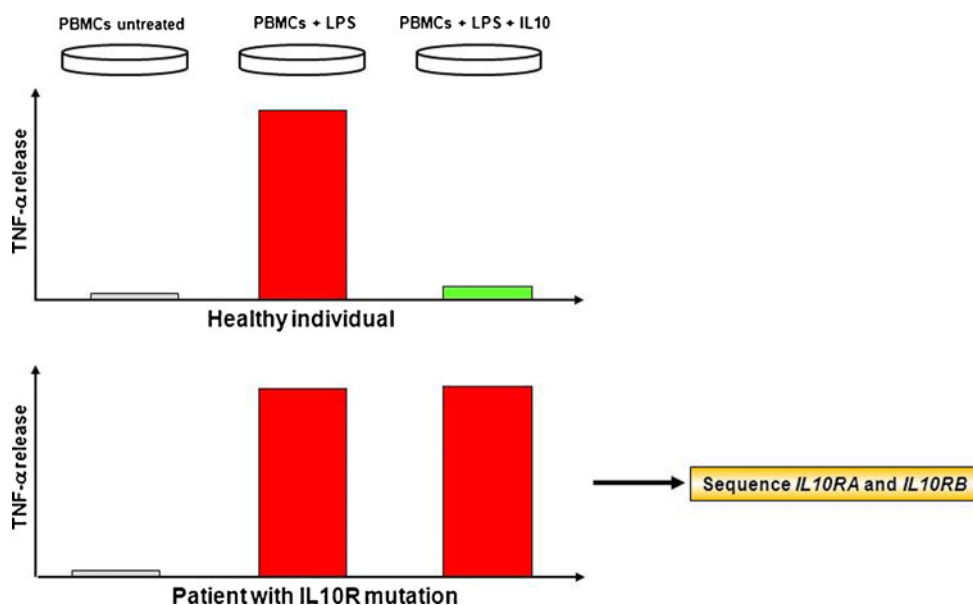
generating docking sites for STAT3. STAT3 then binds to the phosphorylated IL10R1, gets phosphorylated by the receptor associated JAKs and then translocates to the nucleus, where it induces expression of its target genes [61, 62]; this results in inhibition of release of pro-inflammatory mediators such as TNF- α (summarized in Fig. 1) [25].

Both receptor components are required for IL-10 signaling; loss-of-function mutations in either results in a complete signaling failure, which cannot be compensated by any other pathway. IL10R defects can be easily ruled out by functional assays: stimulation of peripheral blood mononuclear cells (PBMCs) from healthy controls with LPS result in the release of several pro-inflammatory cytokines including TNF- α [15•,

18•, 25]. In healthy controls cells, this LPS-mediated release can be entirely inhibited by the addition of exogenous IL-10, but cells from IL10R deficient patients are completely unresponsive to IL-10 (Fig. 2). PBMCs from healthy individuals show strong phosphorylation of STAT3 upon stimulation with IL-10, whereas PBMCs from IL10R-deficient patients do not. Any functional abnormalities are to be confirmed by sequencing the IL10R genes *IL10RA* and *IL10RB* [15•, 16•, 18•].

In patients with wild type IL10R Lack of functional IL-10 due to loss-of-function mutations in the IL-10 gene always needs to be considered. If sequencing of the IL-10 gene reveals putative mutations, *in vitro* synthesis of the mutant IL-10 and subsequent

Fig. 2 Diagnostic procedures to detect IL10R mutations. PBMC from patients with suspected IL10R deficiency are stimulated with LPS and/or co-incubated with IL10; afterwards the release of TNF- α is measured by ELISA. In patients with impaired IL10 signaling, mutations need to be confirmed by sequencing *IL10RA* and *IL10RB*



testing by using STAT3 and/or TNF- α assays is required to prove that the observed mutation is functionally relevant.

With raised awareness and improved availability of genetic testing, even older children might be diagnosed with IL-10 signaling defects. It is, therefore, advisable to screen any patient with history of early onset IBD, fistulizing disease, or unusually aggressive disease progression.

Conclusions

The severe clinical phenotypes in IL-10/IL10R deficiency demonstrate the importance of IL-10 as anti-inflammatory cytokine that controls the chronic stimulation by microbes in the intestine and keeps the immune system in balance; it also shows that in a subgroup of patients IBD may be monogenic and thus distinct from more complex IBD such as UC and CD. IL-10/IL10R deficiency is severe, life-threatening, and resistant to conventional anti-inflammatory drugs, hence, allogeneic HSCT is the only curative therapeutic option. Even though IBD research has very much progressed and our knowledge of the mucosal immunity deepened, there are still many unknown players that need to be identified to help us understand the complex immunity of the intestine.

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