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REVIEW

Maintaining Intestinal Health: The Genetics and Immunology of Very Early Onset Inflammatory Bowel Disease

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SUMMARY

Very early onset inflammatory bowel disease (VEO-IBD) is a distinct form of IBD. Here, we review the current knowledge of the genetic and immunologic basis of VEO-IBD, which requires further investigation for improved and individualized care.

Inflammatory bowel disease (IBD) is a multifactoral disease caused by dysregulated immune responses to commensal or pathogenic microbes in the intestine, resulting in chronic intestinal inflammation. An emerging population of patients with IBD younger than 5 years of age represent a unique form of disease, termed very early onset IBD (VEO-IBD), which is phenotypically and genetically distinct from older-onset IBD. VEO-IBD is associated with increased disease severity, aggressive progression, and poor responsiveness to most conventional therapies. Further investigation into the causes and pathogenesis of VEO-IBD will help improve treatment strategies and may lead to a better understanding of the mechanisms that are essential to maintain intestinal health or provoke the development of targeted therapeutic strategies to limit intestinal inflammation and promote tissue repair. Here, we discuss the phenotypic nature of VEO-IBD, the recent identification of novel gene variants associated with disease, and functional immunologic studies interrogating the contribution of specific genetic variants to the development of chronic intestinal inflammation. *(Cell Mol Gastroenterol Hepatol 2015;1:462–476; http://dx.doi.org/10.1016/j.jcmgh.2015.06.010)*

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Inflammatory bowel disease (IBD), comprising Crohn's disease, ulcerative colitis, and indeterminate colitis, is a multigenetic and environmentally triggered disease resulting in a dysregulated immune response to commensal or pathogenic microbes found in the gastrointestinal tract.[1–7] Patients with IBD exhibit local and systemic immune reactivity to various microbes, have significant alterations in the composition of intestinal commensal bacteria, and can become colonized with pathogenic or opportunistic bacteria.[8–15] The multifactorial nature and environmental contribution to IBD is largely responsible for its increased incidence over the last several decades.[16] Added to the complex nature of the disease, host genetics may play a more prominent role in some subpopulations, particularly in very young children (younger than 5 years of age) in whom the disease is termed very early-onset IBD (VEO-IBD).[17,18] Although this is a heterogeneous population, including some children with mild disease,[19] patients with VEO-IBD can present with a different disease phenotype, including extensive colonic involvement and more severe disease than older children and adults.[17,20,21] In addition, due to poor response to conventional therapies, severity of inflammation, and greater duration of disease, there are higher rates of morbidity in this population.[20,22,23] Because of the aggressive disease phenotype, early age of onset, and strong family history of disease, some measure of VEO-IBD is thought to be a monogenic disease, often involving genes associated with primary immunodeficiencies.[18,20,24] This was elegantly demonstrated with the discovery that several *IL10* (interleukin-10),[25] *IL10RA* (interleukin-10 receptor, α), and *IL10RB* (interleukin-10 receptor, β)[23] gene mutations were associated with a phenotype of severe perianal disease and colitis in infants with VEO-IBD. Additional underlying immunodeficiencies or genetic disorders may also present with an intestinal phenotype in

Abbreviations used in this paper: ADAM17, A disintegrin and metalloproteinase domain 17; CGD, chronic granulomatous disease; COL7A1, collagen, type VII, α1; CVID, common variable immunodeficiency; FOXP3, forkhead box protein 3; GUCY2, guanylate cyclase 2; GWAS, genomewide association studies; IBD, inflammatory bowel disease; IL, interleukin; ILC, innate lymphoid cells; ILC3, group 3 innate lymphoid cells; IgA, immunoglobulin A; IKBKG, inhibitor of κ light polypeptide gene enhancer in B cells, kinase of, γ; IPEX, immunodysregulation, polyendocrinopathy, and enteropathy, X-linked; MHCII, major histocompatibility complex class II; NEMO, nuclear factor-κB essential modulator; RAG, recombination-activating gene; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor; Treg, regulatory T cell; TTC7A, tetratricopeptide repeat domain-containing protein 7A; VEO-IBD, very early onset inflammatory bowel disease; WASP, Wiskott-Aldrich syndrome protein; WES, whole exome sequencing; XIAP, X-linked inhibitor of apoptosis protein.

Most current article

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patients with VEO-IBD.[20,24] These include, but are not limited to, common variable immunodeficiency (CVID), Wiskott-Aldrich syndrome (WAS), immunodysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX) syndrome, and chronic granulomatous disease (CGD).[17,20,22,26]

Studying consanguinity and targeted genetic sequencing has been an extremely valuable approach to allow the identification and characterization of genetic variants associated with VEO-IBD. However, these approaches alone may not identify novel and rare gene variants. Recent advances in sequencing technology such as whole exome sequencing (WES) have broadened our understanding of the pathogenesis of VEO-IBD and resulted in further discoveries of genes and pathways associated with the disease.[26–30] The genomic contribution of IBD has been extensively evaluated through genomewide association studies (GWAS), and over 163 IBD-associated risk loci[31] have been identified.

Several genes located within the IBD-associated loci are critical for regulation of host defense, involving both the innate and adaptive immune responses toward microbes.[31] However, GWAS studies have been primarily performed in adult-onset IBD and in children 10 years of age and older, whose disease is most frequently a polygenic complex disease. Furthermore, GWAS often do not capture rare variants, specifically those with a minor allele frequency of <5%. In contrast, a proportion of patients with VEO-IBD have a monogenic-driven disease or multigenic disease enriched with rare variants of the same or interacting immunologic pathways.[32,23] Thus, as in the case of *IL10RA* and *IL10RB* defects, the development of intestinal inflammation in VEO-IBD patients can be the direct result of defective immune responses.[33]

Although WES has revolutionized our ability to study rare variants and to determine the genetic basis of disease, understanding the relevance of the identified variants has remained challenging. The individual patient's phenotype may be shaped by mode of inheritance, epigenetics, and gene-gene interaction. Environmental modifiers such as the intestinal microbiota, antibiotic exposure, infection, or diet also significantly impact the disease phenotype.[17,26,32] Because of the clinical presentation, often of severe disease, together with the challenge of identifying the unique pathogenesis of the disease, there is currently no standard of care in the evaluation and treatment for VEO-IBD patients. Identifying the driving forces in patients with particularly severe early-onset disease may lead to group-specific therapeutic approaches. Here we discuss the clinical presentation of VEO-IBD patients, the identification of common gene variants associated with the disease, and functional studies that have demonstrated how these variants may contribute to dysregulated immunologic homeostasis in the intestine.

Clinical Presentation of Very Early Onset Inflammatory Bowel Disease

Pediatric IBD has increased in incidence and prevalence, and this phenomenon has included very young children.[16,33,34] VEO-IBD remains relatively uncommon, approximately 6% to 15% of the pediatric IBD population is younger than 6 years old, and disease in the first year of life is rare.[16,34] A subset of patients with VEO-IBD present with a phenotype that is distinct from older children and adults, including extensive colonic disease (pancolitis) in which it is frequently difficult to differentiate ulcerative colitis from Crohn's disease, leading to a diagnosis of indeterminate colitis (Table 1).[20,34] At diagnosis, patients with VEO-IBD are more commonly diagnosed with ulcerative colitis (35% to 59%) as compared to older onset IBD (children older than 6 years and adults) in which Crohn's disease is more prevalent (55% to 60%). In contrast, approximately 30% to 35% of VEO-IBD patients are diagnosed with Crohn's disease. Indeterminate colitis is also diagnosed more often in patients with VEO (11% to 22%) as compared to older onset IBD (4% to 10%).[19,35–37]

Although formal guidelines or standards of care do not exist, disease evaluation of this population includes

Table 1. Features of Very Early Onset and Older Onset Inflammatory Bowel Disease

Feature	VEO-IBD	Older-Onset IBD
Disease distribution		
	Predominately colonic Ileal involvement <20% Extensive disease at presentation	Ileocolonic Less extensive disease at presentation
Disease classification		
	CD: 30%–35% UC: 35%–59% IC: 11%–22%	CD: 55%–60% UC: 40%–45% IC: 4%–10%
Positive family history	40%–50%	10%–20%
Genetic contribution	Increased prevalence monogenic disorders <2 years	Polygenic inheritance
Surgical intervention	~71%	~55%
Other	Therapeutic response to conventional therapy: decreased Consanguinity	

CD, Crohn's disease; IC, indeterminate colitis; UC, ulcerative colitis.

Table 2. Clinical Presentations and Laboratory Abnormalities of Very Early Onset Inflammatory Bowel Disease Patients

Type	Gastrointestinal Manifestations	Laboratory Abnormalities	Pathology
B- and T-cell development			
WAS (WASP)	Microthrombocytopenia Moderate-severe eczema Recurrent or severe infections Colitis: bloody diarrhea	Decreased platelets Low marginal B cells, high transitional B cells, elevated IgA, low IgM Lymphopenia usually present, progressive decline in T cells CD4:CD8 ratio is normal. Natural killer cell abnormalities	Crohn-like inflammatory process: cobblestone appearance and inflammatory pseudo-polyps[38] Ulcerative colitis-like appearance reported: extensive colitis with ulcerations[39] Neutrophil and T_H2 lymphocyte infiltration
Hypogammaglobulinemia, X-lined (BTK) or AR	Diarrhea Chronic infectious diarrhea (*Giardia lamblia*, *Salmonella* species, *Escherichia coli*) Crohn's disease appearance Perirectal abscess and fistula	Hypogammaglobulinemia: defective B-cell maturation secondary to mutations in BTk B cells are severely decreased or absent in the periphery T cells or normal or increased CD4: CD8 ratio is normal or decreased	Lack of plasma cells in lamina propria
Hyper IgM (CD40L, CD40, AICDA, UNG)	Diarrhea, sclerosing cholangitis	Low IgG, IgA Normal or increased IgM Impaired antibody response	
LRBA deficiency	Abdominal pain, diarrhea Hypoalbuminemia may be present	Hypogammaglobulinemia[40]	Small bowel and colon: acute and chronic inflammation can be seen[41]
Hyper IgE syndrome (STAT3, DOCK8)	Susceptibility to infection (staphylococcal infection) Atopic dermatitis Gastrointestinal manifestations most often secondary to infection	Extremely elevated IgE levels Low IgM Decreased memory B cells Neutropenia can be present T cell lymphopenia	Histology and crypt destruction pattern most resembles infectious agent[42,43]
Severe combined immunodeficiency (ZAP70, ITK, LCK)	Severe recurrent infection in infancy Chronic diarrhea, malabsorption, and failure to thrive Oral and esophageal candidiasis	B and T cells are decreased in the peripheral blood T cells may have immature phenotype Variants of severe combined immunodeficiency can have less severe lymphopenia B cells (CD19-HLA-DR) may be normal or decreased Defective IgG, IgA, normal IgM	Hypocellular lamina propria lacking plasma cells or lymphocytes Graft versus host disease-like process in colon or small bowel can be present
X-linked severe combined immunodeficiency (*IL2RG*)	Same	Neutropenia Absence of T cells and natural killer cells Normal B cells	Same
Omenn syndrome (RAG1, RAG2, Artemis, IL7Ra, LIG4, ADA, CHD7)	Diffuse erythroderma Hepatosplenomegaly Lymphadenopathy Chronic diarrhea Failure to thrive	Hypereosinophilia and increased IgE Can have oligoclonal T cells[44] and reduced B cells[45]	Graft versus host disease: numerous apoptotic crypts cells in colon Increase in lamina propria eosinophils[46]

Table 2. Continued

Type	Gastrointestinal Manifestations	Laboratory Abnormalities	Pathology
Common variable immunodeficiency (TAC1, ICOS, CD19, CD20, CD21, CD81)	Heterogeneous presentation Recurrent bacterial infection Chronic infectious diarrhea *Helicobacter pylori* infection common: atrophic gastritis and pernicious anemia48 Malabsorption[48] Granulomatous enteropathy	Decreased memory B cells Defective plasma C ($CD27^+$, CD19, HLA-DR) T cells (CD2, CD3) usually quantitatively normal, T cell abnormalities common CD4:CD8 can be normal or decreased[49]	Antrum: nonspecific increase in lamina propria lymphocytes; apoptotic cells[47] *Helicobacter pylori* positive: atrophic gastritis Small intestine: villous atrophy, increased epithelial lymphocytosis[47] Plasma cells absent or decreased[50]
Epithelial defects			
ADAM 17 deficiency	Present in neonatal period with watery diarrhea that progresses to bloody diarrhea		Hypoplastic crypts in small bowel
Familial diarrhea (GUCY2C)	Neonatal onset of watery diarrhea	Neonatal electrolyte disturbances	Crohn's disease-like appearance
X-lined ectodermal dysplasia and immunodeficiency (IKBKG)	Diarrhea, failure to thrive Susceptibility to infection	B-cell activation defects, can have hypogammaglobulinemia Natural killer cell abnormalities	Enterocolitis with villous atrophy and epithelial shedding[51]
TTC7A deficiency	Severe diarrhea Colitis presentation	Can have low immunoglobulin levels Defect in vaccine response	Small bowel: villous atrophy Colon: apoptosis
Klinder syndrome (FERMT1)	Blistering skin defects and hyperkeratosis of palms and soles	Can have eosinophilia	Intestinal epithelium: focal detachment of epithelium Ulcerations
Dystrophic epidermolysis bullosa (COL7A1)	Bloody diarrhea		Colon: apoptosis Severe colitis
Phagocyte defects			
Chronic granulomatous disease (CYBB: x-linked, CYBA, NCF1, NCF2, NCF4, RAC1)	Recurrent infection/abscesses Perianal disease/fistula Esophageal and gastric outlet obstruction Colitis	Abnormal respiratory burst	Transmural and discontinuous inflammation, with aphthous or serpiginous ulcers can be seen, can be indistinguishable from Crohn's disease[52] Intestinal granulomas, increased compared to Crohn's disease Crohn's disease-like lesions in the small bowel Fistulas, longitudinal ulcers, stenosis Ulcerative colitis appearance Pigmented macrophages
Glycogen storage disease type 1 (SLC37A4)	Crohn's disease presentation Perianal disease and oral manifestations	Neutropenia Neutrophil dysfunction	Perianal disease and oral manifestations may appear
Leukocyte-adhesion deficiency (ITGB2)	Defective chemotaxis, phagocytosis, and bacterial killing Abnormal respiratory burst	Increased peripheral granulocytes	Colonic disease, adhesions, and strictures may be present
Genetic variants in the IL-10/IL-10R pathway and regulatory T cells			
IL-10 ligand and IL10RA and IL10RB	Most frequently neonatal onset of disease, bloody diarrhea Perianal fistula Arthritis Abscesses	Abnormal phosphorylation of STAT3 mediated by IL-10	Ileal and colonic inflammation, ulcerations, inflammatory infiltrates

Table 2. Continued

Type	Gastrointestinal Manifestations	Laboratory Abnormalities	Pathology
X-linked immune dysregulation, polyendocrinopathy, enteropathy (FOXP3, STAT1)	Polyendocrinopathy Recurrent infection Severe enteropathy with watery diarrhea, can be bloody	Lack of $CD4^+$, $CD25^+$, $FOXP3^+$ regulatory T cells Elevated IgA and IgE Normal B cell numbers Eosinophilia	Extensive villous atrophy Features of graft versus host disease with apoptosis of epithelial cells Presence of antienterocyte antibodies along brush border of duodenum[53] Lymphocytic, neutrophilic and eosinophilic infiltration of crypts and crypt abscesses[53] Colitis
Hyperimmune or autoinflammatory			
Mevalonate kinase deficiency (MVK)	Diarrhea Abdominal pain, emesis Recurrent fevers	Hyperimmunoglobulinemia D Natural killer cell dysfunction Elevation of inflammatory markers erythrocyte sedimentation rate, C-reactive protein	Colon: ulcers, cellular infiltrate, apoptosis[54]
Familial Mediterranean fever (MEFV)	Recurrent fevers, diarrhea Abdominal pain Joint pain Amyloidosis Vasculitis Peritonitis Recurrent oral aphthae	Elevated white blood cell count and inflammatory markers	Small bowel inflammation[55] Changes similar to Crohn's disease with chronic inflammation and ulceration may appear[56]
X-linked lymphoproliferative syndrome 1 (XLP1) (SH2D1A), defective SLAM	Epstein-Barr virus triggered hemophagocytic lymphohistiocytosis	Hypogammaglobulinemia Poor antibody response	Unspecified colitis, may have absent plasma cells
X-linked lymphoproliferative syndrome 2 (XLP2, XIAP)	Epstein-Barr virus triggered hemophagocytic lymphohistiocytosis Clinical features similar to observed in Crohn's disease	Hypogammaglobulinemia Decreased natural killer cells can be present	Features similar to Crohn's disease Apoptosis can be seen
Hermansky-Pudlak syndrome (IBD phenotype involvement: HPS1, HSP4, HSP6)	Oculocutaneous albinism Easy bruising Inflammatory bowel disease symptoms of abdominal pain, diarrhea, bloody can be present	Normal platelet count Prolonged bleeding time Platelet dysfunction	Broad ulcers Brown granular pigmentation Nonnecrotizing granulomas

laboratory, radiologic, and endoscopic evaluation (Table 2). A diagnosis at a very young age should trigger concern for a monogenic-driven disease, particularly in IBD diagnosed when the patient is younger than 2 years of age. Furthermore, extensive family history, including a history of disease in male family members (such as in X-linked disease) or a history of infection, skin disease, or autoimmunity can help guide the appropriate laboratory screening. The laboratory studies should include not only the routine screening used for IBD diagnosis but also an immunologic evaluation. This includes vaccine titers, immunoglobulin profiles, analyses of B- and T-cell function, and a dihydrorhodamine flow cytometry assay, and if necessary more targeted phenotyping and functional profiling of the systemic and mucosal immune system. As shown in Table 2, these studies may point to an underlying defect such as neutropenia, which may represent a monogenic disorder causing functional defects in neutrophils such as glycogen storage disease type 1b, leucocyte adhesion deficiency, or congenital neutropenia.

Genetic Variants Associated With VEO-IBD and Their Immunologic Consequences

Monogenic diseases that can present with the phenotype of intestinal inflammation include those that cause defects of intestinal epithelial barrier function, phagocyte bacterial killing, development and function of the adaptive immune system, and hyper- or autoimmune-inflammatory disorders.[32] These genetic alterations may differentially influence the development and progression of intestinal inflammation, so these patients will likely exhibit significant heterogeneity in their responsiveness to therapeutic interventions. We will discuss what we have learned from mouse models and translational patient-based studies, which should be considered when developing therapeutic strategies for these unique patient populations.

Genetic Variants Influencing Intestinal Epithelial Barrier Function

Mutations in genes associated with maintaining integrity of the intestinal epithelial barrier can present with intestinal inflammation in patients with VEO-IBD. These include loss-of-function mutations in *ADAM17* (A disintegrin and metalloproteinase domain 17) resulting in ADAM17 deficiency,[57,58] in *IKBKG* (inhibitor of κ light polypeptide gene enhancer in B cells, kinase of, γ; encoding nuclear factor-κB essential modulator: NEMO) resulting in X-linked ectodermal dysplasia and immunodeficiency,[59] in *COL7A1* (collagen, type VII, α1) resulting in dystrophic epidermolysis bullosa,[60] *FERMT1* (Fermitin family homolog 1) resulting in Kindler syndrome,[61–63] and *TTC7A* (tetratricopeptide repeat domain-containing protein 7A,[29] or gain-of-function mutations in *GUCY2* (guanylate cyclase 2) resulting in familial diarrhea.[17,64] Mutations in these genes may all lead to an impairment of the intestinal epithelial barrier through distinct pathways, such as limiting epithelial regeneration (*ADAM17*),[65] loss of signaling pathways involved in gene expression (*IKBKG*),[66,67] altered cell adhesion, barrier formation, apoptosis (*COL7A1*, *FEMT1*, and *TTC7A*),[29,60–63] or impaired bacterial sensing and ion homeostasis (*GUCY2*).[17,64] The intestinal histology of patients with epithelial defects can be helpful in distinguishing the disease from other causes of intestinal inflammation. For example, patients with *IKBKG* (NEMO) defects may have villous atrophy or epithelial cell shedding on pathologic examination.[68] In contrast, histology in patients with *ADAM17* mutations may demonstrate hypoplastic crypts in the small bowel secondary to a low rate of epithelial production, as *ADAM17* is necessary for transforming growth factor-α to be cleaved from the cell membrane.[69,70]

The intestinal barrier is necessary to maintain a physical separation between commensal bacteria and the mammalian immune system, and a breakdown in this barrier through multiple distinct pathways can directly promote chronic intestinal inflammation.[1,3] In addition to the genes we have listed, intestinal barrier function is maintained through a number of physical and biochemical structures, including mucus production, intestinal epithelial cell tight junction proteins, immunoglobulin A (IgA), and antimicrobial peptides (Figure 1). In mice, chemical disruption of the intestinal barrier through administration of dextran sodium sulfate in drinking water results in dissemination of commensal bacteria and activation of the innate immune system.[71] Chronic exposure to dextran sodium sulfate can lead to activation of the adaptive immune system and to the development of proinflammatory, commensal bacteria-specific B- and T-cell responses,[8,72] which are similar to those observed in IBD patients.[8,73]

Intestinal epithelial cells play an important role in directly regulating immunologic homeostasis in the intestine, as mice with intestinal epithelial cell lineage–specific deletion of factors regulating the nuclear factor-κB pathway, including NEMO and IκB kinase-β, have an increased susceptibility to develop chronic intestinal inflammation.[66,67] Although we know that loss of intestinal barrier function can directly cause intestinal inflammation, additional mouse models and translational patient-based approaches are required to further define how mutations in these genes specifically lead to a breakdown in the barrier, and whether we can develop more targeted therapies to restore barrier integrity and limit chronic inflammation.

Genetic Variants Impairing Development of the Adaptive Immune System

Several genetic variants can alter the development or function of adaptive immune cells in a cell intrinsic or extrinsic manner.[32] Defects that affect development or function of B cells and T cells occur with loss-of-function mutations in recombination-activating genes (*RAG1* or *RAG2*) or the IL-7R (*IL7R*) causing Omenn syndrome, or the *PTEN* (phosphatase and tensin homolog) gene causing PTEN syndrome. Defects in *RAG1*, *RAG2*, or *IL-7R* can cause cell-intrinsic defects in the development of both T cells and B cells by blocking either early lymphocyte survival or

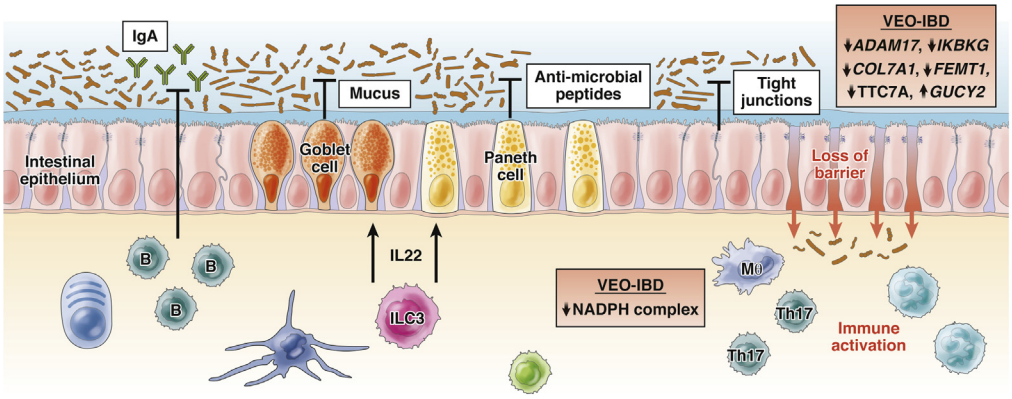

Figure 1. Intestinal epithelial barrier function plays an essential role in maintaining intestinal health. Physical and biochemical barriers, including tight junctions, immunoglobulin A, antimicrobial peptides, mucus, and the ILC3-IL-22 pathway, maintain intestinal epithelial barrier function. This is essential to maintain anatomic segregation between commensal bacteria and the mammalian immune system. Loss of this physical segregation can promote dysregulated innate and adaptive immune cell responses. Identified genetic variants that result in a loss or gain of function mutation and are associated with very early onset inflammatory bowel disease are noted in orange boxes.

recombination of the B-cell receptor or T-cell receptor.[74–76] Defects in B-cell development lead to an absence of circulating mature B cells and antibody production, which have been linked to an IBD phenotype.[77] This includes agammaglobulinemia, which can also occur in X-linked agammaglobulinemia[78] and CVID, a complex and heterogeneous disease, with the responsible mutations known for only a minority of cases.[79] Recently, one candidate gene causing CVID and potentially contributing to intestinal inflammation was identified using WES as a loss-of-function mutation in *LRBA* (lipopolysaccharide-responsive beige-like anchor), resulting in multiple defects in immune cell populations.[40]

Related to CVID, antibody deficiencies associated with IBD manifestations include IgA deficiency and severe combined immunodeficiency, which can be secondary to multiple variants that influence the development or function of the adaptive immune system (including *RAG1, RAG2, JAK3, CD45, CD3G, ZAP70, ADA, DCLRE1C*).[32,77,80] Omenn syndrome, a recessive form of severe combined immunodeficiency, involves abnormal development of B cells and T cells, and can also be associated with intestinal disease as well as severe eczematous rash.[80,81] In these patients, laboratory studies indicate increased oligoclonal T cells and reduced B cells, and histologic examination can show an intestinal graft versus host appearance.[82,83] Conversely, overproduction of specific immunoglobulins, such as hyper IgM, hyper IgE syndrome (resulting from a loss of function mutation in *DOCK8*) can also result in intestinal inflammation and an IBD phenotype.[84]

It is currently unclear exactly how these selective impairments of the adaptive immune system can manifest in intestinal inflammation. There is a potential involvement of altered regulatory pathways or chronic infections with pathogenic and opportunistic microbes. Therefore, additional lines of study are required to further interrogate the link of these mutations to intestinal inflammation.

Wiskott-Aldrich syndrome results from a loss-of-function mutation in the Wiskott-Aldrich syndrome protein (*WASP*), and patients can exhibit thrombocytopenia, eczema, immune deficiencies, and intestinal inflammation.[85] The clinical manifestation of VEO-IBD patients with this genetic defect can be pancolitis in addition to other autoimmune processes. *WASP* is a critical cytoskeleton protein expressed in hematopoietic cells, and it is required for the normal development and function of multiple cell types.[86,87] *WASP* is critical for peripheral B-cell development and function and thus the ability to respond to antigens.[88,89] Laboratory studies in these patients may show thrombocytopenia, low IgM levels, low marginal B cells, and lymphopenia.[90] Nguyen et al[91] identified that intestinal inflammation in *WASP*-deficient mice was critically dependent upon inflammatory T cells, which may result from impaired development of regulatory T cells (Tregs) in the thymus and periphery.[92] Surprisingly, these defects are likely occurring in a cell-extrinsic manner, as the absence of *WASP* in cells of the innate immune system directly contributed to the development of inflammatory T-cell responses in mice.[93]

The causes of intestinal inflammation in other similar patient populations are less well understood, but defects in regulatory T cells, IgA, and abnormal selection of T-cell and B-cell specificities likely contribute. Additional immunologic analyses and mouse models, such as those we have described, are needed to define the causes of disease further and to develop potential therapeutic options in these patient populations.

Genetic Variants Impairing Regulatory T Cells

Defects in regulatory T cells can clinically present as colonic disease as well as an enteropathy. IPEX syndrome is most often secondary to mutations of the forkhead box protein 3 (*FOXP3*) gene, a transcription factor that is essential for the development and immunosuppressive

activity of CD4 Foxp3^{+} Tregs.[94,95] There are over 20 mutations in *FOXP3* that have been identified in patients with IPEX,[96] and patients frequently present with neonatal severe secretory diarrhea, failure to thrive, infection (due to defects in immunoregulation), skin rash, insulin-dependent diabetes, thyroiditis, cytopenias, and other autoimmune disorders.[94,95] Tregs are absent or dysfunctional in these patients, and in the intestine histologic analyses may reveal infiltration of inflammatory cells in the lamina propria and submucosa of the small bowel and colon as well as changes in the mucosa of the small bowel.[94] Other genetic defects have been found to cause IPEX-like disease, including loss-of-function mutations impacting IL-2/IL-2R interactions, STAT5b (signal transducer and activator of transcription 5b), and ITCH (itchy E3 ubiquitin protein ligase), or gain-of-function mutations in STAT1, all of which critically influence the development and function of Tregs.[81] Further, Zeissig et al[97] have recently identified in VEO-IBD a novel loss of function mutation in *CTLA4* (cytotoxic T lymphocyte-associated protein 4), a surface molecule of regulatory T cells that directly suppresses effector T-cell populations.

The mechanisms by which regulatory T cells limit intestinal inflammation are well characterized in mice. Tregs can develop in the thymus as "natural Tregs" and directly contribute to limiting proinflammatory T cells in the intestine.[98] The composition of commensal bacteria influences the repertoire of Tregs,[98] and commensal bacteria-specific "induced Tregs" can also be generated in the periphery after sampling of commensal bacteria by dendritic cells in the intestine and migration to the mesenteric lymph node (Figure 2).[1,5,99,100] Once generated, Tregs can then promote intestinal homeostasis through direct regulation of innate and adaptive immune cell responses to commensal bacteria, a process that involves cytokine production, direct cell-cell contact (in part through CTLA4), and sequestering of growth factors.[1,5,99]

Consistent with a major role for Tregs in limiting proinflammatory immune cell responses to commensal bacteria, mice deficient in IL-2 or FoxP3 develop significantly less intestinal inflammation when maintained in germ-free versus conventional housing conditions, but they exhibit comparable levels of systemic autoimmunity.[101,102] Recent evidence also suggests that the balance of tissue-specific IL-23 and IL-33 expression in mice is critical for regulating the function of Tregs in the intestine and chronic inflammation,[103] although the role of these pathways in human VEO-IBD has not been examined.

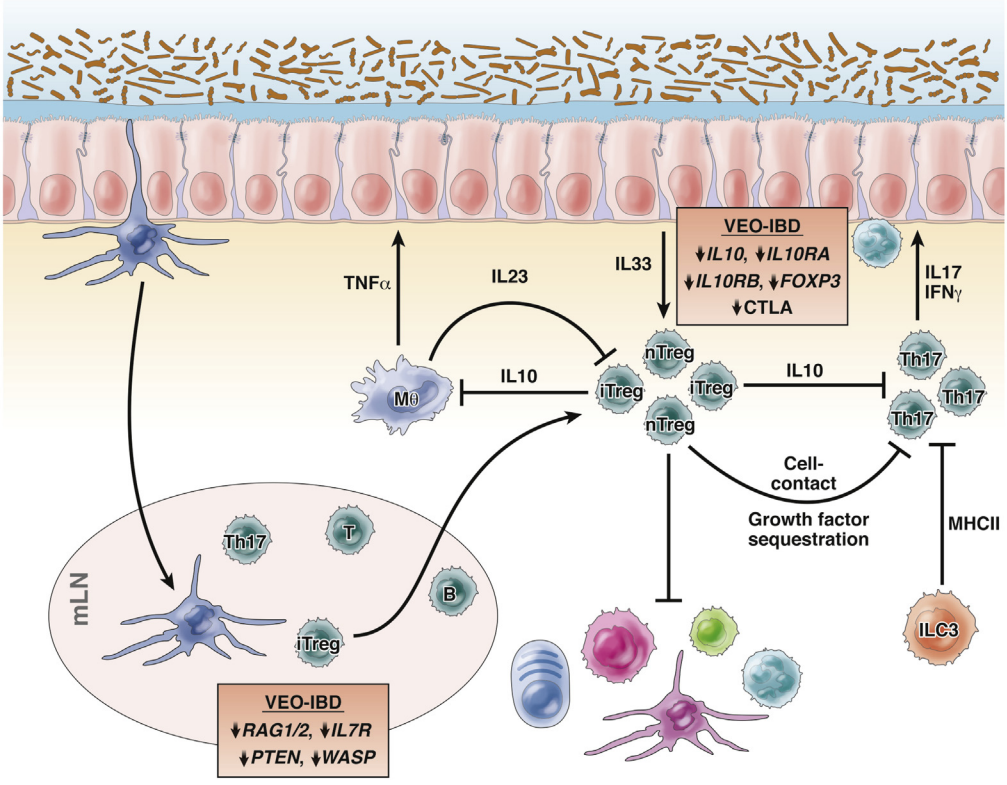

Figure 2. Regulatory T cells, interleukin-10 (IL-10) and ILC3 critically limit dysregulated immune responses to commensal bacteria. Regulatory T cells (Treg) can differentiate in the thymus or periphery and limit immune cell responses to intestinal commensal bacteria through multiple mechanisms, including cytokine production, direct cell-to-cell contact and sequestering of growth factors. Further, IL-10 production by multiple cell types can directly promote anti-inflammatory responses from myeloid cells to limit intestinal inflammation. ILC3 can also directly limit proinflammatory T cells through MHCII-dependent interactions. iTreg, inducible regulatory T cell; nTreg, natural regulatory T cell. Identified genetic variants that result in a loss or gain of function mutation and are associated with very early onset inflammatory bowel disease are noted in orange boxes.

Genetic Variants in the Interleukin-10/Interleukin-10 Receptor Pathway and Related Cytokine Family Members

Homozygous loss of function mutations in *IL10* ligand and receptors *IL10RA* and *IL10RB* are associated with significant intestinal inflammation, particularly in neonatal or infantile VEO-IBD, with a phenotype of severe enterocolitis and perianal disease.[23,25] In addition, compound heterozygote loss of function mutations of *IL10RA* have been reported with neonatal Crohn's disease and enterocolitis.[104] IL-10 is an anti-inflammatory cytokine secreted by a variety of cells, including dendritic cells, natural killer (NK) cells, eosinophils, mast cells, macrophages, B cells and $CD4^+$ T cell subsets (including T_H2 cells, T_H1 cells, T_H17 cells and Treg).[105,106] IL-10 maintains homeostasis through suppression of an excessive proinflammatory response and exerts its effect through binding to the IL-10 receptor, IL-10R, which is a tetrameric complex.[107] It is composed of 2 distinct chains, 2 molecules of IL-10R1 (α chain) and 2 molecules of IL-10R2 (β chain).[108] IL-10 binding to IL-10R activates the JAK1/STAT3 cascade, which subsequently limits proinflammatory gene expression.[108] In addition to intestinal inflammation, IL-10 defects are associated with arthritis, folliculitis, and a predisposition to lymphoma.[104,109] Given that the defects in IL-10-IL-10R interactions predominantly influence the immune system, a potential treatment for these patients is successful hematopoietic stem cell transplantation.[110] Although this can be challenging and typically requires an HLA-identical donor, there has been recent success reported with haploidentical stem cell transplantation, however nonengraftment complications can occur.[111]

An essential role for IL-10 in limiting intestinal inflammation was demonstrated when IL-10 deficient mice were generated and found develop severe spontaneous colitis,[112] and subsequent studies by Sartor et al[113] identified that the intestinal inflammation in IL-10-deficient mice was entirely dependent upon the presence of commensal bacteria. Therefore, IL-10 plays a critical role in limiting dysregulated immune cell responses to intestinal commensal bacteria (Figure 2). The exact cellular sources and targets of IL-10 that contribute to the maintenance of intestinal homeostasis have been less well defined until the recent development of mice that permit conditional deletion of IL-10 and IL-10R. These critical studies have revealed an essential role of regulatory T cell-intrinsic IL-10 expression in preventing intestinal inflammation in mice.[114,115] Further, it was recently demonstrated that IL-10R expression on myeloid cells in mice is critical to elicit anti-inflammatory responses and limit T cell-dependent intestinal inflammation.[116,117] Critically, patients with loss-of-function mutations in *IL10RA* or *IL10RB* also exhibited an impaired ability to differentiate anti-inflammatory myeloid cells in vitro, and rather exhibited increased proinflammatory properties, such as elevated expression of IL-6, IL-12, tumor necrosis factor-α (TNFα), MHCII (major histocompatibility complex class II), and costimulatory molecules.[116] Although mouse models have provided invaluable insight into human health and disease, it should be noted that mice deficient in Il10 do not completely replicate the phenotypes of humans with loss-of-function mutations in IL10, likely due to many confounding factors.

IL-22 is a cytokine that is related to IL-10, shares the IL-10R2 chain with a unique IL-22R1, signals through predominantly STAT3, and also plays a critical role in mediating intestinal homeostasis.[118] However, unlike IL-10, the functional IL-22R is restricted to predominantly non-hematopoietic cells; in the intestine, IL-22 acts almost exclusively on intestinal epithelial cells to mediate innate immunity and intestinal barrier function (Figure 1).[118] IL-22 can be produced by T_H17 cells, and more recently has been identified to be predominantly expressed by a previously unrecognized cell type of the innate immune system, termed group 3 innate lymphoid cells (ILC3).[118,119] This breakthrough in immunology has led to the identification of other members of the innate lymphoid cell (ILC) family, including group 1 ILCs that express T-bet and proinflammatory cytokines TNFα and interferon-γ, and group 2 ILCs that express GATA3 and type 2 cytokines IL-4, IL-5, IL-9, IL-13, and amphiregulin.[119,120]

The ILC family exhibits a heterogeneity comparable to that of differentiated CD4 T-cell subsets, and plays a profound role in regulating intestinal health and disease in mouse models.[118–120] Critically, recent reports suggest that ILC3 is a dominant source of IL-22 in the intestine of healthy humans, and that dysregulated ILC responses are observed in adult patients with IBD.[121–127] Further, we have also recently identified that ILC3 expresses MHCII, and that selective deletion of MHCII on ILC3 results in dysregulated CD4 T-cell responses and spontaneous intestinal inflammation.[122] $MHCII^+$ ILC3 selectively induces cell death of proinflammatory, commensal bacteria-specific CD4 T cells in the intestine; critically, we observed that MHCII was reduced on ILC3 from intestinal biopsy tissues of pediatric IBD patients versus non-IBD controls, and that this was inversely correlated with the level of proinflammatory T_H17 cells.[128]

Despite these advances, ILC and IL-22 responses have yet to be explored in VEO-IBD. Given the importance of these pathways in mediating intestinal health and disease, it is likely that the genetic variations associated with VEO-IBD, such as IL7/IL7R, may differentially influence ILC responses.

Genetic Variants Influencing Bacterial Recognition and Clearance

Chronic granulomatous disease (CGD) is a result of defective intestinal phagocytes, specifically the granulocytes responsible for bacterial killing and clearance.[129] The NADPH oxidase complex is responsible for killing ingested microbes through its production of the respiratory burst. Mutations in any part of the complex molecules (CYBB, CYBA, NCF1, NCF2, NCF4) can result in intestinal inflammation as well as autoimmune disease.[130,131] Intestinal inflammation can be observed in as many as 40% of patients with CGD.[52,132–134]

Several variants have been associated with VEO-IBD, in particular defective NCF2 (neutrophil cytosolic factor 2)

results in altered binding to RAC2 (ras-related C3 botulinum toxin substrate 2).[135] These patients can present in the neonatal or first year of life with colitis, severe fistulizing perianal disease, and stricturing.[135] Histology frequently demonstrates multiple granulomas that may not have associated inflammatory change.[26] Critically, a recent study by Dhillon et al[136] identified that heterozygous loss-of-function mutations in components of the NADPH oxidase complex can determine susceptibility to VEO-IBD, without directly causing overt immunodeficiency. Other neutrophil defects that are associated with VEO-IBD include leukocyte-adhesion defect due to mutation in ITGB2 (integrin, β2).[137,138] These patients can present with an IBD phenotype, history of bacterial infection, and laboratory studies remarkable for increased peripheral granulocytes.[139] Glycogen storage disease type 1b, with hallmark features of neutropenia and neutrophil granulocyte dysfunction, can present with intestinal inflammation.[140]

The reasons why CGD and other bacterial processing defects may manifest in intestinal inflammation are poorly understood and warrant additional research. It is possible that the causes include bacterial overgrowth or dysbiosis in the intestine, dysregulated activation of the innate and adaptive immune system, or both.

Further, the therapies used to treat such patients need to be carefully considered. For example, anti-TNFα therapy is contraindicated in CGD; although it is effective for intestinal disease, it can increase the risk of severe infections in these patients.[141] Other therapies include leukine, antibiotics, and allogenic hematopoietic stem cell transplantation, which have demonstrated some success.[142] Recent evidence suggests that IL-1R antagonists may be a particularly attractive approach to limit disease in mouse models and patients with CGD by restoring autophagy and directly limiting inflammation.[143]

Hyper- and Autoimmune-Disorders

Several autoimmune diseases have been linked with intestinal inflammation in children with VEO-IBD. These include mevalonate-kinase deficiency,[144] familial Mediterranean fever (FMF),[145,146] Hermansky-Pudlak syndrome,[147] and X-linked lymphoproliferative syndrome (types 1 and 2).[28,148,149] These diseases occur due to loss-of-function mutations in an enzyme critical for metabolism (mevalonate-kinase deficiency), cytoskeletal proteins (familial Mediterranean fever), proteins involved in organelle fusion or biogenesis (Hermansky-Pudlak syndrome), or proteins involved in cell signaling or apoptosis (X-linked lymphoproliferative syndrome). Although there are many additional clinical manifestations in these patients, 20% of patients with X-linked lymphoproliferative syndrome that have a loss-of-function defect in the gene X-linked inhibitor of apoptosis protein (*XIAP*), present with VEO-IBD.[150]

XIAP is involved in nucleotide-binding oligomerization domain-containing protein 2 (NOD2)-mediated nuclear factor-κB signaling, so these children may have an impaired ability to sense bacteria. In addition, as an inhibitor of apoptosis, it prevents apoptosis of activated T cells, thus allowing for expansion and survival of T cells in response to pathogens.[151,152] Therefore, in XIAP deficiency, due to the inability to clear pathogens, there is a hyperinflammatory state with increased production of cytokines resulting in an IBD phenotype.[150,152] Children with these mutations can present with severe colonic and perianal fistulizing disease;[28,153] of great concern, Epstein-Barr virus infection can result in fatal hemophagocytic lymphohistiocytosis.[153]

This was not an exhaustive description of the rare genomic drivers of VEO-IBD, but it highlights the different components of the immune system, including innate and adaptive responses, involved in this disease. Treatments guided toward the specific defect, such as IL-1 antagonists, colchicine, hematopoietic stem-cell transplantation, or leukine can be used if the defect is determined. Additionally, monitoring for potential complications associated with a genetic defect is essential, such as in XIAP, IL-10 gene variants, and CGD. In addition to these monogenic diseases, VEO-IBD has been shown to have a high degree of genetic heterogeneity. It is therefore likely that there are more pathways involved in VEO-IBD, and the outcome of therapeutic intervention can be improved through further study and identification of the associated variants. Using next-generation sequencing technology such as WES can improve the detection of variants and the diagnosis of disease. Further, there is an urgent need to also directly translate genes to function and to functionally profile the immunologic significance of known genetic variations in intestinal inflammation.

Perspective and Future Directions in Genetic and Immunologic Analyses of Very Early Onset Inflammatory Bowel Disease

To advance our understanding of VEO-IBD, new sequencing technology must be used to completely survey the genetic landscape of this disease. Immunologic studies spanning basic mouse models and translational patient-based approaches are required to determine the contribution of those genetic variations to human disease. Given that dysregulated interactions between the immune system and commensal bacteria underlie the pathogenesis of intestinal inflammation, it is also important to include analyses of the composition and function of the microbiome. Given that these patient populations are studied worldwide and sometimes in small numbers, an international registry containing the genetic, immunologic, and environmental data of VEO-IBD patients could prove beneficial in our goal of better understanding the effects of different variants within known genes and identifying new gene defects that cause IBD through the study of mutations that arise in the same genes of multiple unrelated individuals. With an increased understanding of the disease processes operating in VEO-IBD, we can begin to individualize therapies to the specific patient or patient groups, as well as employ unconventional therapies that are not routinely part of the IBD therapeutic arsenal. These approaches could provide a

roadmap to establishing a standard of care for this disease and improving patient quality of life.

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Conflicts of interest
The authors disclose no conflicts.

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