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REVIEW ARTICLE

# Intestinal immunoregulation: lessons from human mendelian diseases

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The mechanisms that maintain intestinal homeostasis despite constant exposure of the gut surface to multiple environmental antigens and to billions of microbes have been scrutinized over the past 20 years with the goals to gain basic knowledge, but also to elucidate the pathogenesis of inflammatory bowel diseases (IBD) and to identify therapeutic targets for these severe diseases. Considerable insight has been obtained from studies based on gene inactivation in mice as well as from genome wide screens for genetic variants predisposing to human IBD. These studies are, however, not sufficient to delineate which pathways play key nonredundant role in the human intestinal barrier and to hierarchize their respective contribution. Here, we intend to illustrate how such insight can be derived from the study of human Mendelian diseases, in which severe intestinal pathology results from single gene defects that impair epithelial and or hematopoietic immune cell functions. We suggest that these diseases offer the unique opportunity to study in depth the pathogenic mechanisms leading to perturbation of intestinal homeostasis in humans. Furthermore, molecular dissection of monogenic intestinal diseases highlights key pathways that might be druggable and therapeutically targeted in common forms of IBD.

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## INTRODUCTION

The intestinal barrier must be a permeable surface able to efficiently absorb nutrients and to precisely regulate water and electrolytes exchanges. Conversely, this barrier must cope with the huge load of symbiotic microbes and resist invasion by pathogens. In order to reconcile these contradictory functions and to preserve host fitness, the intestinal barrier has evolved a complex partnership between epithelial cells and hematopoietic immune cells. The mechanisms underlying the establishment and the maintenance of the intestinal barrier have been extensively explored over the past 20 years in mice inactivated for genes expressed in epithelial cells, in hematopoietic cells, or in both.[1] In many instances, impairment of the epithelial barrier was only evidenced after perturbing the steady state, either by using a chemical agent such as dextran sulfate[2] or by introducing microbes endowed with pathogenic traits, such as *Helicobacter hepaticus*,[3] illustrating both the redundancy of the mechanisms which maintain gut homeostasis and the role of environmental triggers in altering local regulations. In humans, comparable complexity of the intestinal barrier can be inferred from genome wide studies in inflammatory bowel diseases (IBD), which pinpoint over 230 predisposing genetic loci.[4] Yet, neither studies in mice, nor studies in multifactorial forms of human IBD in which environmental factors play a determinant role, allow identifying key nonredundant mechanisms and hierarchizing their respective contributions to the human intestinal barrier. Herein, we intend to illustrate how such insight can be derived from the study of human Mendelian diseases, in which severe intestinal pathology results from alteration in single gene. We do not intend to review exhaustively all monogenic intestinal disorders but rather to focus on emblematic gene defects that impair the functions of epithelium, of hematopoietic immune cells, or both and discuss whether and how they can promote intestinal inflammation. A few non-monogenic antibody defects that can be associated with intestinal inflammation are also discussed.

## MENDELIAN DISORDERS IMPAIRING INTESTINAL EPITHELIAL CELLS

Schematically, they can be divided into three categories whether they affect digestion/absorption, epithelial differentiation, or epithelial innate defense (Table 1).

### Diseases impairing epithelial transport functions

A first group of rare and generally autosomal recessive disorders arise from mutations in genes coding for transporters or enzymes located in the small intestinal brush border (reviewed in[5,6]). They manifest by congenital diarrhea (defined as starting at or shortly after birth) that can be life threatening but is usually the main or only symptom and contrasts with normal intestinal histology. Treatment relies on rehydration and specifically adapted diets. The lack of intestinal inflammation in most defects suggests a clear dichotomy between the digestive and immune functions of the gut barrier. The edge is, however, not sharp. Thus, intestinal inflammation has been observed in some patients with congenital chloride diarrhea caused by loss-of function (LOF) mutations in SLC26A3, a solute carrier (also called DRA for down-modulated in adenoma) that controls epithelial $Cl^-/HCO3^+$ exchange in ileum and colon[7] (Fig. 1a). Interestingly, a variant predisposing to

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**Table 1.** IBD-like disorders associated with causal or predisposing variants.

| Class | Defects | Gene | Inheritance | OMIM # | Gut phenotype |
|---|---|---|---|---|---|
| Disorders impairing intestinal epithelial cells | Epithelial transport | *SLC26A3* | AR | 126650 | Congenital chloride diarrhea |
| | | *GUCY2C* | AR/AD | 601330 | Congenital sodium diarrhea |
| | | *SLC9A3* | AR | 182307 | |
| | Enterocyte differentiation and or polarization | *MYO5B* | AR | 606540 | Microvillus inclusion disease |
| | | *STX3* | AR | 600876 | |
| | | *STXBP2* | AR | 601717 | Microvillus inclusion disease/ hemophagocytic lymphohistiocytosis |
| | | *EPCAM* | AR | 185535 | Epithelial dysplasia/tufting enteropathy |
| | | *SPINT2* | AR | 605124 | |
| | Enterocyte differentiation | *WNT2B* | AR | 601968 | Congenital diarrhea |
| | Epithelial innate defense | *NOX1* | XLR/risk factor | 300225 | IBD-like disease |
| | | *DUOX2* | AR/AD/ risk factor | 606759 | |
| | | *ALPI* | AR | 171740 | |
| Disorders impairing both epithelial and hematopoietic immune cells | Epithelial and lymphocyte differentiation | *TTC7A* | AR | 609332 | IBD-lke disease or intestinal atresia and immunodeficiency |
| | | *TTC37* | AR | 614589 | Congenital diarrhea and immunodeficiency |
| | | *SKIV2L* | AR | 600478 | |
| | NF-κB signaling | *IKBKG* | XLR | 300248 | Immunodeficiency and colitis not cured by HSCT |
| Disorders impairing effector function of innate hematopoietic immune cells | NADPH oxidase | *CYBB* | XLR | 300481 | Chronic granulomatous disease and IBD-like disease |
| | | *CYBA* | AR | 608508 | |
| | | *NCF1* | AR | 608512 | |
| | | *NCF2* | AR | 608515 | |
| | | *NCF4* | AR | 601488 | |
| | NOD2-CARD15 signaling | *XIAP* | XLR | 300079 | Crohn's disease like |
| | | *TRIM22* | AR | 606559 | IBD-like disease |
| | | *NPC1* | AR | 607623 | Lysosomal lipid storage disease ± IBD-like disease |
| Disorders impairing intrinsic regulation of innate hematopoietic immune cells | Intrinsic check-points of NF-κB cascade | *TNFAIP3* | AD | 191163 | Multiorgan inflammation and autoimmunity |
| | | *OTULIN* | AR | 615712 | |
| | Intrinsic check-points of inflammasomes | *NLRC4* | AD | 606831 | Macrophage activation syndrome, neonatal enterocolitis |
| | | *MVK* | AR | 251170 | Periodic fever syndrome, neonatal enterocolitis |
| | Intrinsic regulation of apoptosis or necroptosis | *RIPK1* | AR/AD | 603453 | IBD-like disease |
| | | *CASP8* | AR | 601763 | |
| Disorders impairing extrinsic regulation of hematopoietic immune cells | IL-10 signaling | *IL10* | AR | 124092 | Colitis and perianal lesions |
| | | *IL10RA* | AR | 146933 | |
| | | *IL10RB* | AR | 123889 | |
| | TGF-β signaling | *TGFB1* | AR | 190180 | Colitis |
| | | *TGFBR1* | AD | 190181 | IBD-like disease, eosinophilic diseases, and allergy |
| | | *TGFBR2* | AD | 190182 | |
| Disorders impairing activation and regulation of adaptive immune cells | X-linked agammaglobulinemia | *BTK* | XLR | 300300 | B-cell deficiency, IBD-like disease |
| | B-cell ± T-cell differentiation or activation | *ICOS* | AR | 604558 | CVID, IBD-like disease |
| | | *TNFRSF13C* | AR, risk factor | 606269 | |
| | | *TNFRSF13B* | AD, risk factor | 604907 | |
| | | *CD27* | AR | 186711 | |
| | | *IL21* | AR | 605384 | |
| | | *IL21R* | AR | 605383 | |
| | | *CD19* | AR | 107265 | |
| | | *MS4A1* | AR | 112210 | |
| | | *CR2* | AR | 120650 | |
| | | *CD81* | AR | 186845 | |

**Table 1.** continued

| Class | Defects | Gene | Inheritance | OMIM # | Gut phenotype |
|---|---|---|---|---|---|
| | | *PRKCD* | AR | 176977 | |
| | | *PLCG2* | AD | 600220 | |
| | | *NFKB1* | AD | 164011 | |
| | | *NFKB2* | AD | 164012 | |
| | | *PIK3CD* | AD | 602839 | |
| | | *RAG1* | AR | 179615 | Omenn syndrome, intestinal inflammation |
| | | *RAG2* | AR | 179616 | |
| | Regulatory T-cell differentiation or activation | *FOXP3* | XLR | 300292 | IPEX syndrome, autoimmune enteropathy |
| | | *IL2RA* | AR | 606367 | IPEX-like syndrome, autoimmune enteropathy, infections |
| | | *IL2RB* | AR | 146710 | |
| | | *STAT5B* | AR | 604260 | |
| | | *CTLA-4* | AD | 123890 | Multiorgan autoimmunity, autoimmune enteropathy, CVID |
| | | *LRBA* | AR | 606453 | |
| | Hyperactivation of JAK-STAT signaling | *STAT3 GOF* | AD | 102582 | |
| | | *STAT1 GOF* | AD | 600555 | Multiorgan autoimmunity, autoimmune enteropathy |
| | | *JAK1 GOF* | AD | 147795 | |
| | | *PTPN2* | AD | 176887 | |

*AR* autosomal recessive, *AD* autosomal dominant, *CVID* common variable immunodeficiency, *HSCT* hematopoietic stem cell transplantation, *IPEX* immunodysregulation polyendocrinopathy enteropathy X-linked.

ulcerative colitis and mapping in the *SLC26A3* region was found in the Japanese population.[8] How SLCA26A3 deficiency might predispose to intestinal inflammation is unclear. Possible hypotheses include alteration of the mucus layer by the diarrheal flux and or change in microbiota composition induced by the acidic luminal pH, although firm evidence is still lacking (Fig. 1a). Strikingly, Crohn-like inflammation has also been reported in several patients with congenital sodium diarrhea caused either by recessive LOF mutations in *SLC9A3*[9] or by dominant gain of function (GOF) in *GUCY2C*.[10,11] *SLC9A3* encodes the sodium proton antiporter 3 (NHE3), an apical $Na^+/H^+$ exchanger that is key for $Na^+$ absorption and for acid-base homeostasis,[9] while GUCY2C is an intestinal guanylate cyclase transmembrane receptor converting GTP in cyclic GMP. GUCY2C enzymatic activity is triggered either by bacteria-derived heat-stable enterotoxins or by guanylin and uroguanylin, two endogenous high avidity ligands produced by epithelial cells. By increasing cyclic GMP intracellular concentrations, GUCY2C elicits a complex signaling cascade implicating protein kinases, which ultimately stimulates chloride secretion by the cystic fibrosis transmembrane conductance regulator (CFTR), while simultaneously reducing sodium absorption through NHE3 (reviewed in[12]) (Fig. 1b). This function explains the secretory diarrhea observed in patients with *GUCY2C* GOF mutations and, conversely, the neonatal obstruction by meconial ileus observed in a few Bedouin children with LOF mutations.[13] The proinflammatory role of GUCY2C is less well understood. Interestingly, *gucy2c*$^{-/-}$ mice display reduced sensitivity to DSS colitis,[14] while, in contrast, *Slc9a3*$^{-/-}$ mice deficient in NHE3 develop spontaneous distal colitis with neutrophil infiltration.[15] In the latter mice, reduced acidification at the luminal surface was suggested to increase bacterial adherence to the mucosa and thereby to trigger inflammation, a hypothesis supported by the curative effect of oral treatment with metronidazole and ciprofloxacin.[15] A comparable mechanism may operate in patients with *GUCY2C* GOF mutations. Finally, the study of *gucy2c*$^{-/-}$ mice suggested a role of GUCY2C in the negative control of epithelial cell proliferation[12] (Fig. 1b). Excessive activation of GUCY2C may thus also impair epithelial repair and thereby promote intestinal inflammation.

Diseases impairing enterocyte differentiation and polarization

By preventing electrolyte and nutrient absorption, these diseases result in severe congenital diarrhea. Intestinal inflammation is variable, depending on the underlying mechanisms, but develops with time in many cases, attesting the need of an impervious physical epithelial barrier to maintain local immune homeostasis.

*Microvillus inclusion disease (MVID)*. This emblematic disease, first described in 1978,[16] is characterized by atrophy of the apical brush border membrane and intracellular accumulation of microvilli lined inclusion bodies, which can be visualized by periodic acid-Schiff or CD10 staining and by electron microscopy. Epithelial architecture is impaired with often severe villous atrophy. While MVID typically manifests as congenital diarrhea, a few patients develop clinical symptoms and histological inflammation, which can be misdiagnosed with very early onset inflammatory bowel diseases (VEO-IBD). Thus, in our cohort, LOF *MYO5B* mutations were identified by next generation sequencing in a 3-year-old boy, initially diagnosed with very early onset Crohn-like disease.[17] LOF biallelic mutations have been successively identified in *MYO5B*,[18] *STX3*,[19] and *STXBP2*,[20] three genes that encode functionally and spatially related proteins orchestrating apical trafficking in enterocytes (reviewed in[21]). Most mutations affect *MYO5B*, which encodes the actin-based molecular motor myosin VB. This protein interacts with the Rab11 and Rab8A GTPAses, which control the transport of endosomes and vesicles to the brush border. Accordingly, Myosin 5B-deficient enterocytes display a severe polarization defect (reviewed in[22]). Lack of apical expression of the SLC9A3/NHE3 and SLC26A3/DRA transporters that are necessary for $Na^+$ and $Cl^-$ absorption (see above) contrasts with normal CFTR expression, altogether explaining the severe sodium chloride diarrhea[23–25] (Fig. 1c). Strikingly, *MYO5B*-deficient cells also display relocalization from the apical to the intracellular compartment of two other proteins that can be found mutated in MVID, the target soluble NSF attachment protein (t-SNARE) syntaxin 3 encoded by *STX3* and syntaxin-binding protein 2 (also called Munc18-2) encoded by *STXBP2* (reviewed in[21]). Both proteins form a complex essential for

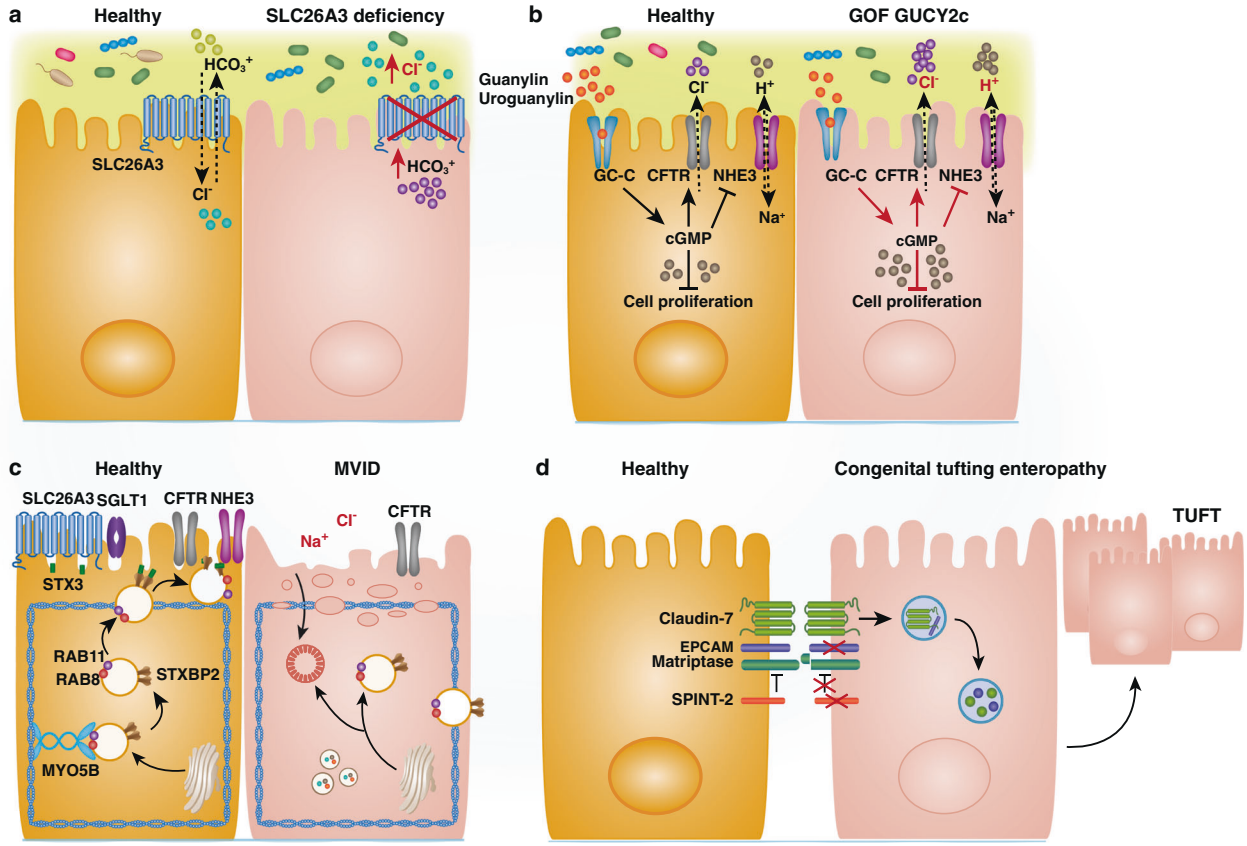


**Fig. 1 Mechanisms of barrier defect in diseases impairing epithelial apical transport or differentiation.** LOF mutations in the solute carrier SLC26A3 (**a**) or GOF mutations in the guanylate cyclase transmembrane GUCY2C (**b**) result in severe congenital diarrhea which may alter mucus, intraluminal pH, and microbiome composition. Excessive production of cGMP due to hyperactivation of GUCY2C can not only inhibit NH3-dependent absorption of sodium but also stimulate chloride secretion via CFTR (cystic fibrosis transmembrane conductance regulator) and impair epithelial repair, overall promoting intestinal inflammation. In microvillus inclusion disease (MVID) (**c**), LOF mutations in *MYO5B*, *STXBP2*, or *STX3* prevent transport (*MYO5B*) to or fusion of vesicles (*STXBP2* or *STX3*) containing brush border proteins with the enterocyte apical pole, thus impairing brush border formation and epithelial polarization. Mis-localization of NHE3, SLC26A3, and SGLT1 (cotransporter glucose-Na+) but not of CFTR results in severe sodium chloride diarrhea. In congenital tufting disease (**d**), lack of EPCAM at the basolateral membrane prevents its interaction with claudin 7. Alternatively, lack of HAI2 encoded by *SPINT2* results in excessive activation of matriptase which cleaves EPCAM, thereby allowing lysosomal targeting of EPCAM and claudin 7. In both defects, loss or reduced expression of EPCAM and claudin 7 impairs formation of intercellular or cell-to-matrix junctions with loss of epithelial sealing, formation of tufts, and likely increased bacterial translocation.

membrane fusion of vesicles at the enterocyte brush border, thus explaining how LOF mutations may cause MVID[20] (Fig. 1c). *STXBP2* mutations are, however, best known for causing familial hemophagocytic lymphohistiocytosis, a very severe inflammatory disorder that results from lack of macrophage control by cytotoxicity-defective lymphocytes.[26] Indeed Munc18-2 plays a key role in T and NK lymphocytes where, by interacting with syntaxin 11, it regulates exocytosis of cytotoxic granules.[26] While hematopoietic stem cell transplantation (HSCT) can prevent acute macrophage activation, it does not improve diarrhea in *STXBP2* defective patients with MVID, indicating that the epithelial defect develops independently of the immune disease.[27]

The MVID phenotype has been recently recapitulated in the enterocytes of *myo5b*$^{-/-}$ mice[24,28] and pigs,[25] of *stxbp2*$^{-/-}$ mice as well as in organoids derived from *myo5b*$^{-/-}$ [28] and *stxbp2*$^{-/-}$ [29] mice, allowing elegant mechanistic studies. Typical microvillus inclusions were shown to appear late in organoids during the maturation phase of enterocytes[29] and to result from excessive apical bulk endocytosis[28] as well as from ectopic basal internalization of mislocalized microvilli.[29] Importantly, genetic inactivation of Pacsin 2 in *myo5b*$^{-/-}$ mice inhibited apical bulk endocytosis and reduced the number of microvillus inclusions but failed to restore brush border expression of transporters. It is therefore likely that the loss of apical transporters is not due to excessive brush border endocytosis, but rather results from defective apical trafficking.[28] Finally, recent studies showed that lysophosphatidic acid could improve brush border maturation and transporter trafficking both in vitro in *myo5b*$^{-/-}$ organoids and in vivo in *myo5b*$^{-/-}$ mice.[30] Moreover, lentiviral transduction of the normal human *STXBP2* gene into *stxbp2*$^{-/-}$ mouse organoids could restore enterocyte phenotype,[29] overall indicating that organoids are promising tools to test novel therapeutic options that are badly needed for this very severe epithelial disease.

*Epithelial dysplasia or congenital tufting enteropathies (CTE).* CTE form a second group of very severe diseases disrupting epithelium differentiation and integrity, which can be complicated by intestinal inflammation. The characteristic lesions are foci of crowding enterocytes or "tufts," present in both small and large intestines. The small intestine displays villous atrophy of variable severity. Mononuclear cell infiltration can be important and mimic an inflammatory disease, notably when tufts are not prominent or not recognized.[31] Biallelic LOF mutations were first identified in *EPCAM*, which encodes the epithelial cellular adhesion molecule, a 42 kD transmembrane protein that is most strongly expressed at the latero-basal membrane of enterocytes and allows $Ca^{++}$-independent homophilic interactions.[32] A minority of CTE patients carry mutations in *SPINT2*, which encodes a membrane type

2 serine protease inhibitor called HAI.[33] *Epcam*$^{-/-}$ mice developed an intestinal disease recapitulating human CTE that was lethal within 1 week after birth.[34] Multiple alterations of the intestinal epithelial structure and barrier functions have been described in EPCAM-deficient enterocytes, including mis-localization of E-cadherin in the intracellular compartment that impairs the formation of adherent junctions,[35] brush border alterations,[36] and also desmosome abnormalities with decreased latero-basal expression of the α2β1 integrin and of claudin 7.[31,34] Indeed, this unusual claudin is not only present in tight junctions but is also strongly expressed at the latero-basal membrane of enterocytes, where it was found to interact physically with EPCAM.[37] Strikingly, *Cldn7*$^{-/-}$ mice died within 10 days of life from severe CTE-like disease with extensive inflammation.[38] Based on these observations, Wu et al.[39] provided compelling evidence for a key role of claudin 7-EPCAM interaction in CTE, with HAI2 playing a regulatory role. Mechanistically, they showed that claudin 7 and EPCAM are reciprocally stabilized by their association, which is likely necessary for maintaining optimal cell-to-cell and cell-to-matrix adhesion. This association is regulated by proteases and notably matriptase, which cleaves EPCAM into a 36kD fragment unable to bind claudin 7 so that, upon dissociation, EPCAM and claudin 7 are directed toward lysosomal degradation. Importantly, matriptase activity is negatively regulated by HAI2 encoded by *SPINT2*. As a consequence, matriptase is abnormally active in the enterocytes of patients with *SPINT2* LOF mutations, resulting in excessive degradation of both EPCAM and claudin 7 and thereby in CTE[39] (Fig. 1d). Disease expression is exclusively digestive in *EPCAM*-deficient patients, except for rare cases of arthritis, perhaps related to the loss of intestinal barrier integrity. This phenotype contrasts with the broad distribution of EPCAM across epithelia but may be explained by the absence in the gut of TROP2, an EPCAM homolog expressed in most other epithelia (discussed in[40]). In contrast, *SPINT2*-deficient patients often display extra-digestive symptoms, notably punctuated keratitis and malformations such as esophageal and or choanal atresia and imperforated anus, the mechanism(s) of which remain(s) unexplained.[33]

*Deficiency in WNT2B*. Severe impairment of gastrointestinal differentiation associated with histological evidence of inflammation was reported recently in three children with homozygous nonsense *WNT2B* mutations, who developed intractable diarrhea of neonatal onset.[41] WNT2B is a potent agonist of the Wnt/β-catenin pathway that plays a central role in proliferation and maintenance of intestinal stem cells. Secreted by a subset of pericryptic mesenchymal cells as a palmitoylated protein, WNT2B can engage Frizzled receptors and their Lrp5/6 coreceptors at the surface of adjacent stem cells, causing stabilization and nuclear accumulation of β-catenin and subsequent induction of a transcriptional program that drives proliferation (reviewed in[42]). Based on studies in organoids, WNT2B was proposed to be an extraepithelial source of Wnt able to compensate for the absence of WNT3, another strong agonist of Wnt signaling that is secreted by Paneth cells.[43] The lack of pathology in *wnt2b*$^{-/-}$ mice[44] further supported a redundant role with WNT3.[43] In contrast, paucity of crypts and crypt architectural defects in gastric, duodenal, and colonic biopsies of WNT2B-deficient children indicates that WNT2B plays a nonredundant role in the maintenance of the gastrointestinal stem cell niche in humans, a conclusion supported by the reduced number of olfactomedin 4-expressing stem cells in biopsies and by decreased mRNA expression of some WNT targets in enteroids.[41] Strikingly, addition of recombinant WNT2B failed to sustain prolonged growth of enteroids as well as to rescue colonic or gastric organoids,[41] suggesting that the absence of WNT2B severely impaired intestinal stem cell survival. The impact of WNT2B deficiency on epithelial barrier and the mechanism(s) underlying the chronic inflammation observed in biopsies remain, however, elusive, as well as the long-term prognosis of this severe epithelial disease.

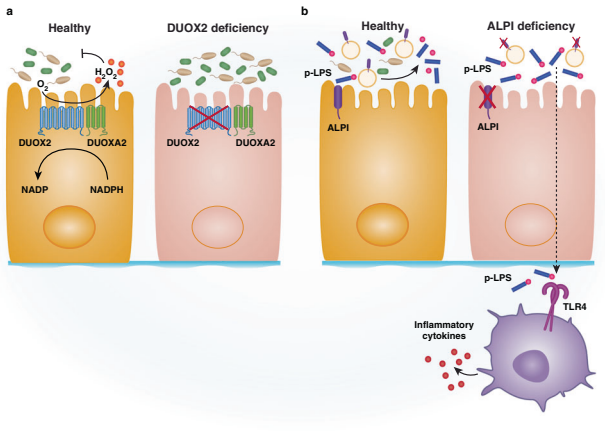


**Fig. 2 Defects impairing innate epithelial defenses.** The NADPH oxidase DUOX2 is transcriptionally induced by microbes via MyD88 or TRIF signals. Upon binding with its maturation factor DUOXA, it is transported at the intestinal epithelial surface where it transforms $O_2$ in microbicide $H_2O_2$. Lack of DUOX2/DUOXA can promote bacterial colonization and translocation and thereby promote intestinal inflammation (**a**). Intestinal alkaline phosphatase (ALPI) is produced in the small intestine and released at the surface of vesicles in the lumen where it can hydrolyze phosphate residues from LPS lipid A and thereby reduce its agonist activity on TLR4. Lack of ALPI results in LPS-TLR4-dependent intestinal inflammation (**b**).

Defects impairing innate epithelial defenses

*Epithelial nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and intestinal inflammation*. A considerable redundancy between the 1540 human genes thought to encode for innate immune functions has been recently suggested as <6% have been associated with primary immunodeficiencies.[45] Accordingly, very few clinically overt diseases have been associated with deficiencies in single innate immune genes. Among exceptions are, however, neutrophil oxidase 2 (NOX2), the NADPH oxidase that is expressed in phagocytes (see below) and, although at a lesser extent, NOX1 and DUOX2, two NADPH oxidases that are expressed in the human gut epithelium. NOX1, which converts $O_2$ into superoxide $O_2$, is a multi-subunit complex with a catalytic core made of two transmembrane proteins, p22phox and NOX1, and three cytosolic subunits. DUOX2, which produces $H_2O_2$, forms a membrane-bound heterodimer with its maturation factor DUOXA (reviewed in[46]) (Fig. 2a). Very rare monoallelic LOF missense variants in *NOX1* (encoded by the X chromosome) and *DUOX2* have been reported in several children developing IBD before 6 years.[47–49] More recently, we have identified biallelic *DUOX2* mutations impairing $H_2O_2$ generation in a child with very severe colitis starting at 3 years.[50] In drosophila, DUOX inactivation led to uncontrolled colonization by a pathogenic proteobacterium,[51] while inactivation of DUOXA in mice, which prevents DUOX2 surface expression, resulted in increased bacterial translocation and upregulation of epithelial chemokines.[52] Epithelial $H_2O_2$ production thus appears to be one important conserved innate immune mechanism that participates in microbiota control and intestinal homeostasis (Fig. 2a). Of note, in the *DUOX2*-deficient child, heavy immunosuppression was necessary to control intestinal inflammation, but only during the first 2 years following diagnosis,[50] suggesting that compensatory mechanisms may operate when DUOX2 is inactive. A rescue role of lactobacillus-derived $H_2O_2$ was suggested.[53] NOX1 and DUOX2 may also exert partially redundant functions in the production of reactive oxygen species (ROS) at the epithelial surface.[53]

Finally, NOX2-dependent production of ROS by phagocytes may counterbalance defective epithelial NADPH oxidase activity (see below). Overall, *NOX1* and *DUOX2* mutations should rather be considered as IBD risk factors, as disease penetrance appears to be low.[46,48]

*Intestinal alkaline phosphatase (ALPI) and intestinal inflammation*. Strikingly, there is no evidence that deficiency in individual Toll-like receptors (TLR), or in MyD88 or IRAK4 can affect intestinal homeostasis. Thus, the few patients carrying mutations in such genes exclusively displayed very narrow and transient susceptibility to extra-intestinal infections.[54] However, the recent identification of LOF mutations in ALPI as a cause of VEO-IBD indicates that the activation of TLR and notably of TLR4 must be tightly regulated to maintain intestinal homeostasis.[55] ALPI exerts its phosphatase catalytic activity on several substrates and notably on lipopolysaccharides (LPS), one major component of the membrane of gram-negative bacteria. By removing one of the two phosphate moieties present in LPS, ALPI can reduce by 1000-fold their TLR4 agonist activity, thus providing a potent anti-inflammatory mechanism.[56] ALPI is expressed by enterocytes in the small intestine and released from the brush border into the intestinal lumen at the surface of lipid vesicles, where it is protected from degradation, thus allowing its delivery as a functional enzyme in the colon (reviewed in[57,58]) (Fig. 2b). Using whole-exome sequencing, Parlato et al.[55] have recently identified biallelic mutations inactivating ALPI expression and activity in two unrelated children, who had developed ileal and or colonic inflammation refractory to all medical treatments. This observation in humans extends previous studies in zebrafish and in mouse, which demonstrated the key protective role of ALPI against TLR4-dependent intestinal inflammation[59,60] (Fig. 2b). Altogether they provide a rationale for oral ALPI supplementation in patients with IBD, in which fecal concentrations of ALPI are decreased (reviewed in[55,58]).

## MENDELIAN DISEASES IMPAIRING BOTH EPITHELIAL AND HEMATOPOIETIC IMMUNE CELLS

These diseases might impair differentiation and or signaling (Table 1).

### Defects affecting both epithelial and immune cell differentiation

A first disease results from mutations in *TTC7A* that encodes the tetratricopeptide repeat (TPR) domain 7A protein. Affected children variably display severe enterocolitis or/and multiple intestinal atresia, thymus alterations, and progressive T, B, and NK lymphopenia resulting in high susceptibility to a broad range of pathogens but also to autoimmune diseases.[61–64] TTC7A is highly expressed in hematopoietic and epithelial cells where, via its 9 TPR domains, it functions as multiprotein scaffold in several cellular compartments. In enterocytes, TTC7A deficiency is associated with increased apoptosis, impaired actin-cytoskeleton rearrangement, and inversion of apicobasal polarity. These alterations have been ascribed to the role of TTC7A in recruiting phosphatidylinositol 4-kinase IIIa at the plasma membrane[61] and in inhibiting RhoA kinase activity.[62] Accordingly, a rho kinase inhibitor could rescue epithelial alterations in patient derived intestinal organoids,[62,64] while leflunomide was identified in a drug screen as able to reduce apoptosis of *TTC7A*-deficient human epithelial cells and to restore gut abnormalities in *ttc7a*$^{-/-}$ zebrafish.[64] The antiapoptotic effect of leflunomide was associated with restoration of phosphorylation and activation of AKT, a crucial survival kinase that is activated by phosphatidylinositol kinases.[64]

A second disease called the tricho-hepato-enteric syndrome results from autosomal-recessive mutations in either TTC37, another TPR containing protein, or in the RNA helicase SKIV2L. The two proteins belong to the heterotetrameric Ski complex involved in mRNA decay and homeostasis. Patients develop severe congenital diarrhea with lesions of villous atrophy and variable inflammation in small and large intestine, immunodeficiency with hypogammaglobulinemia and poor vaccinal responses but also impairment of T- and NK cell functions, liver fibrosis or cirrhosis, and typical hair defects ([65] and reviewed in[66,67]). The underlying mechanism is unknown. Inactivation of SKIV2L has been associated with activation of RIG-1-like receptors and overproduction of type I interferon in response to activation of the endoplasmic reticulum stress response.[68] Yet, this was not observed upon inactivation of TTC37. As mutations in both *TTC37* and *SKIV2L* give rise to a strictly overlapping syndrome,[68] the role of type I interferon in disease pathogenesis remains quite uncertain. Overall, mutations in *TTC7A*, *TTC37*, and *SKIV2L* lead to severe defects in epithelial differentiation, resulting in leaky gut barrier with consequences that may be aggravated by the associated immune deficiency. In contrast, mutations in NEMO discussed below affect innate immune functions of both epithelial and hematopoietic cells.

### Defective activation of NF-kB signaling in epithelial and hematopoietic immune cells

Severe intestinal inflammation is one frequent symptom in patients with hypomorphic mutations in X-linked *IKBKG* gene, which encodes IKK-γ (also called NEMO for NFκB-essential modulator), the regulatory subunit of IκB kinase (IKK). IKK-γ/NEMO is indispensable for activating the kinase function of the two catalytic subunits IKK-α and IKK-β and thereby, for triggering the canonical NFκB pathway. Indeed, IKK phosphorylates NFκB inhibitor (IκB) at sites that trigger its ubiquitination and degradation, thus releasing NFκB, which can translocate into nucleus and induce transcription of its target genes[69,70] (Fig. 3a). Complete *NEMO* inactivation is lethal, which is not surprising given the multiple pathways converging into the canonical NFκB pathway (including TLR, IL-1/18 and tumor necrosis factor (TNF) receptors, T- and B-cell receptors, CD40, FAS, RANK, vascular endothelial receptor-3, and ectodysplasin A).[70,71] Hypomorphic mutations in male patients lead to a complex syndrome that variably combines ectodermal dysplasia (with skin, hair, and teeth abnormalities), severe bacterial infections, osteopetrosis, lymphangiomas, and autoimmune and inflammatory disorders.[70,71] Severe inflammatory colitis is observed in 20–50% of patients.[71,72] Defective NFκB signaling in hematopoietic immune cells impairs their activation and can thereby compromise the gut barrier. In addition, NEMO deficiency impairs epithelial intrinsic functions. Thus, selective inactivation of NEMO in mouse IEC led to apoptosis of colonocytes, impaired defensin production, and bacterial translocation. As a consequence, immune hematopoietic cells were hyperactivated and induced severe chronic inflammation, which could be prevented by blocking MyD88 or TNFR signaling.[73] The later result is in keeping with the fact that, in absence of NEMO, TNF activation leads preferentially to apoptosis (reviewed in[70]). Overall, these data explain the poor outcome of HSCT in NEMO-deficient patients. Thus, HSCT reconstituted immune cells in most cases, but it failed to cure colitis in several patients, and was even followed by de novo appearance of colitis in others[71] (Fig. 3b).

More recently, LOF mutations in other components of the NFκB pathway, namely RIPK1[74] and caspase 8,[75] have been associated with severe immunodeficiency and colitis. Yet, while selective inactivation of either molecules in mouse IEC have been associated with severe epithelial apoptosis and necroptosis,[76,77] the mechanism of intestinal lesions seems different in humans and may rather result from excessive activation of the inflammasome in immune hematopoietic cells. The consequences of these mutations will therefore be discussed below with genetic diseases that impair exclusively or predominantly the hematopoietic compartment of the gut barrier.

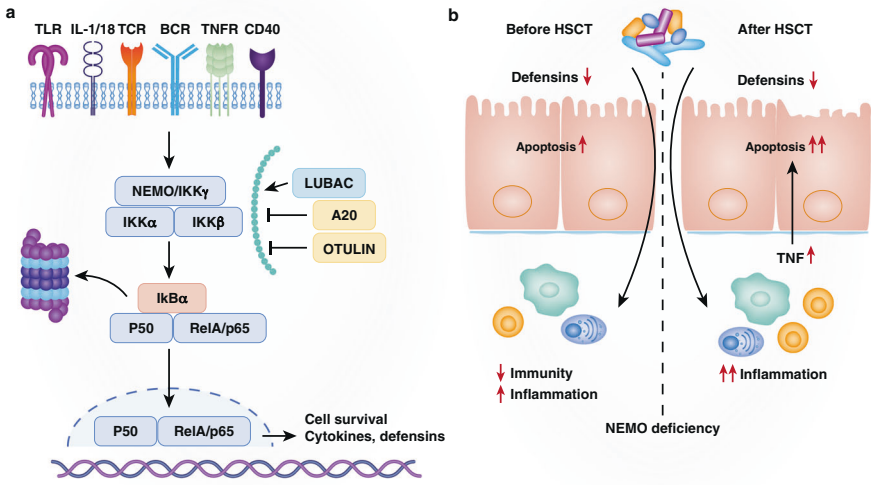


**Fig. 3 Mechanism of colitis in NEMO hypomorphic mutations.** NEMO, the regulatory subunit of the IkB kinase activates canonical NF-kB signaling downstream multiple receptors. Complete loss is lethal. **a** Hypomorphic mutations impair effector and regulatory functions of immune hematopoietic cells but also epithelial cell survival and innate immune functions, notably their production of microbicide peptides. Colitis is thereby a frequent complication. Hematopoietic stem cell transplantation (HSCT) restores NF-kB signaling in hematopoietic immune functions but not in epithelial cells. Following HSCT, microbial translocation can trigger activation of intestinal macrophages and their production of TNF which, in absence of NF-kB signals, further stimulates epithelial cell death and can sustain or even trigger colitis (**b**).

## DISEASES AFFECTING THE HEMATOPOIETIC COMPARTMENT OF THE GUT BARRIER

A broad spectrum of single gene defects impairing either the effector or the regulatory functions of immune cells can compromise intestinal homeostasis. Strikingly, disease expression varies depending whether the defect affects innate or adaptive immune cells. In keeping with data derived from GWAS in common multifactorial IBD,[78] monogenic defects that alter predominantly innate immune responses and notably responses to the microbiota tend to affect in priority the distal parts of intestine and notably the colon. This is illustrated below by the case of mutations that impair the effector functions of phagocytes or, conversely, result in their excessive activation. In contrast, gene defects impairing adaptive immunity and its regulation preferentially manifest in the small intestine and are often associated with autoimmunity and adverse responses to food proteins (Table 1).

Defects impairing effector functions of innate immune cells

*Chronic granulomatous disease (CGD) and intestinal inflammation*. In CGD, the lack of functional NADPH oxidase complex in neutrophils and monocyte/macrophages impairs microbial clearance, resulting in life-threatening bacterial and fungal infections. In addition, ~50% patients develop intestinal inflammation (reviewed in[79,80]). NADPH oxidase comprises five subunits. The glycoprotein gp91$^{phox}$ or NOX2 encoded by the *CYBB* gene on the X chromosome, and four other subunits encoded by autosomal genes, p22$^{phox}$ encoded by *CYBA*, and p47$^{phox}$, p67$^{phox}$, and p40$^{phox}$ encoded by *NCF1*, *NCF2*, and *NCF4*, respectively. About 70% of the CGD patients are males carrying hemizygous LOF mutations in *CYBB*; 20 and 5% carry LOF recessive mutations in the autosomal genes *NCF1* and *NCF2*, respectively.[79,80] Mutations in *NCF4* are rarer and manifest with peripheral rather than invasive infections and with inflammatory lesions of the skin and gastrointestinal tract.[81] The mechanisms by which NADPH oxidase enables microbial killing by phagocytes have been largely unraveled. Two proteins, gp91$^{phox}$ (the catalytic- and phagocyte-specific subunit) and p22$^{phox}$ (shared by other NOX complexes), form a membrane heterodimer called cytochrome $b_{558}$ at the surface of resting phagocytes. The other subunits p40$^{phox}$, p47$^{phox}$, and p67$^{phox}$ are cytosolic and form a heterotrimer, which associates with cytochrome $b_{558}$ upon cell activation, resulting in a conformational change of p91$^{phox}$. This change initiates the binding of electrons in the cytosol and their transport to the cell surface, where they reduce $O_2$ into superoxide $O_2^-$. This compound is delivered at the cell surface or into phagosomes engulfing microbes. $O_2^-$ can then be transformed into hydrogen peroxide ($H_2O_2$) and in other more reactive compounds such as hypochlorous acid ($HOCl^-$) by myeloperoxidase in neutrophils or reactive nitrogen species by inducible nitric oxide synthase in activated macrophages (Fig. 4a). ROS can directly contribute to microbial killing (reviewed in[79]). They can also cause $K^+$ influx into phagosomes, which raises pH and allows the release and activation of neutrophil granule proteases.[82] Finally, ROS may stimulate the activation of NETosis, a variety of neutrophil death that leads to the formation of extracellular stretches of chromatin containing granular proteases, in which microbes can be entrapped and killed.[83,84] Overall these data explain how the lack of functional NOX2 can lead to severe and recurrent bacterial and fungal infections. In addition, ~70% CGD patients develop inflammatory manifestations and, notably, 30–60% develop Crohn-like disease with diarrhea and severe colonic inflammation containing granulomas within the first decade of life. Perineal ulcerative lesions or abscesses are frequent and other parts of the gastrointestinal tract can be affected, notably the ileum (reviewed in[79]). As in common IBD, patients frequently display serum anti-saccharomyces cerevisiae antibodies. Intestinal inflammation can develop in CGD patients without prior evidence of infections, justifying to test NADPH oxidase function in all patients with early onset Crohn-like disease. Of note, PMA-induced dihydrorhodamine-1,2,3 oxidation, which is broadly used to test NADPH oxidase activity is not impaired by NCF4 deficiency, and demonstration of the functional defect requires to test particle-induced NADPH oxidase activity in neutrophils.[81] Because of the striking overlap between the digestive expression of CGD and clinical and histological features in patients with very early onset colitis, Dhillon et al. have used targeted next generation sequencing to

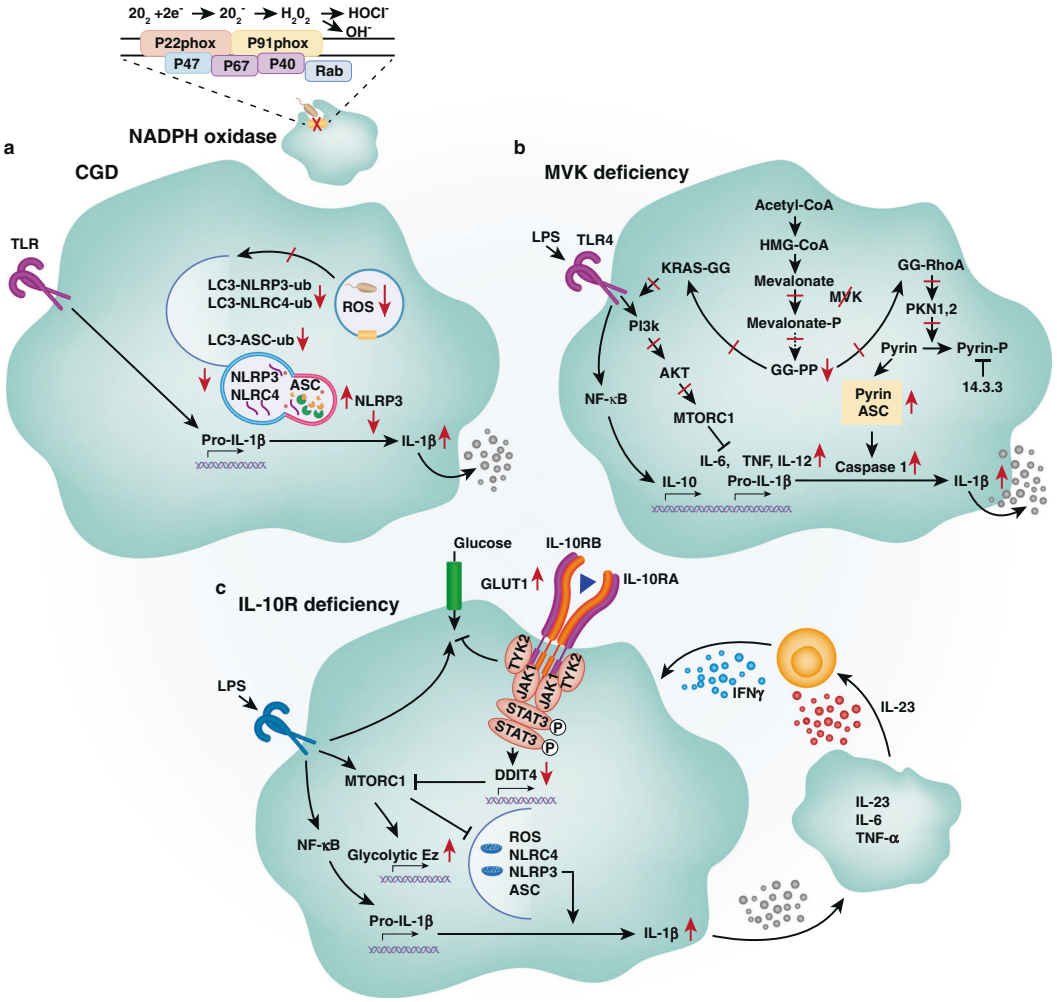


**Fig. 4 Mechanisms leading to inflammasome activation and intestinal inflammation in diseases impairing macrophage effector functions or regulation.** In chronic granulomatous disease (CGD) (**a**), LOF mutations in the subunits of the NADPH oxidase NOX2 impair production of reactive oxygen species (ROS) and thereby recruitment of microtubule-associated protein 1 light chain 3 (LC3) to autophagosomes, resulting in less degradation of inflammasome components NLRP3, NLRC4, caspase 1 and excessive activation of the inflammasome. In mevalonate kinase deficiency (MVKD) (**b**), defective production of geranyl–geranyl pyrophosphate (GG-PP) prevents activation of KRAS and downstream activation of PIK3cδ (PI3K) and mTOR that inhibits TLR-induction of inflammatory cytokines, notably pro-IL-1β. Lack of GG-PP also impairs activation of RhoA and thereby that of PKN kinases that maintain the pyrin inflammasome in its phosphorylated inactive form. As a result, pyrin can detach from its inhibitor 14.3.3, assemble with apoptosis-associated speck-like protein containing a CARD (ASC) triggering caspase 1 activation and pro-IL-1β cleavage into mature IL-β (**b**). In LPS-activated macrophages, IL-10 (**c**) inhibits glycolysis (see text) and induces transcription of DDIT4, which inhibits mTOR and thereby licenses autophagy and degradation of inflammasome components. IL-10R deficiency therefore prevents their degradation, enhancing conversion of pro-IL-1β into mature IL-β. In turn, IL-β stimulates production of inflammatory cytokines, notably IL-23, by a distinct subset of inflammatory monocytes. IL-23 can next activate T-cell production of IFNγ, which further activates monocytes and macrophages, creating a vicious circle.

screen 122 children developing colitis before 6 years of age for mutations in the phagocyte NADPH oxidase and in Rac1 and Rac2, two GTPases which contribute to NADPH oxidase activation. These authors identified 11 variants present at the heterozygous state, which may impair interactions between subunits of the NADPH oxidase complex and reduce their expression or ROS production. Yet, the five children who could be tested showed NADPH oxidase activity that was low but within normal range and the exact significance of these variants remains uncertain.[85]

The mechanisms driving inflammation in CGD patients remain incompletely understood. Several non-mutually exclusive hypotheses have been suggested, including an inhibitory role of ROS on T-cell or innate cell activation[79] or the stimulation of the type I interferon pathway which was connected with the propensity of CGD patients to develop autoimmunity and lupus-like symptoms.[86] Another attractive hypothesis relies on the role of NOX2-generated ROS in autophagy and more specifically in promoting the recruitment of microtubule-associated protein 1 light chain 3 to the phagosome[87] (Fig. 4a). Accordingly, autophagy is abnormal in murine and human cells defective in NADPH oxidase.[88] In keeping with the role of autophagy in degrading inflammasome components and inhibiting inflammasome activation (reviewed in[89]), NADPH oxidase-deficient cells produced increased amounts of IL-1β[88] (Fig. 4a). Accordingly, IL-1β blockade with Anakinra decreased severity of TNBS-induced colitis in $p47^{phox-/-}$ mice and induced clinical remission or improvement in two CGD patients with refractory colitis.[88] This beneficial effect of Anakinra was, however, not confirmed in five additional patients.[90] Of note and in contrast with anti-TNF treatment, which results in life-threatening infections and notably in liver abscesses in CGD patients, blocking IL-1β did not increase the risk of infections in the seven Anakinra-treated patients.[88,90]

*Mendelian diseases of the NOD2-CARD15 pathway and intestinal inflammation*. Twenty years after the first identification of *NOD2* exonic variants as the main genetic risk factor for Crohn's disease[91,92] and despite numerous studies highlighting how NOD2 may contribute to multiple pathways involved in intestinal homeostasis, including defensin secretion, pro- or anti-inflammatory cytokine production, antigen presentation, autophagy-dependent bacterial clearance, the exact role of NOD2 remains uncertain.[93] Moreover, *nod2*$^{-/-}$ mice do not develop spontaneous intestinal inflammation. Yet, strikingly, severe intestinal inflammation has been observed in patients carrying mutations inactivating two NOD2-interacting partners, the X-linked inhibitor of apoptosis (XIAP) and the protein encoded by the tripartite motif-containing gene 22 (TRIM22).

*XIAP* mutations were initially identified as one cause of hemophagocytic lymphohistiocytosis (HLH) induced by Epstein–Barr virus (EBV). Further studies revealed that ~25% of XIAP-deficient patients developed severe Crohn-like disease that was usually refractory to medical treatment (reviewed in[94]). Of note, intestinal inflammation can develop in *XIAP*-deficient patients without HLH and at very variable age, from a few weeks or months after birth to over 40 years even in the same family, pointing to the highly variable penetrance of the disease and to the probable role of environmental factors. This X-linked disease affects mainly boys. Yet, several cases were observed in heterozygous female carriers: they were ascribed to the selective inactivation of the unaffected X chromosome in macrophages.[95] Mechanisms behind HLH and colitis are thought to involve different domains of XIAP. XIAP comprises three baculovirus inhibitor of apoptosis repeats (BIR) domains, a ubiquitin-associated domain (UBA), and a C-terminal RING domain with E3 ubiquitin-ligase activity. Via its BIR2 and three domains, XIAP can potently inhibit caspases 3, 7, and 9. EBV-induced HLH might therefore result from excessive apoptosis of XIAP-deficient T, NK, or NKT cells, which fail to eliminate activated macrophages (reviewed in[94]). On the contrary, the role of XIAP in IBD was linked to a second function downstream of NOD1 and NOD2, which depends on the BIR2 domain and on the ubiquitin-ligase activity of the RING domain.[96–98] Daamgard et al. thus showed that, upon activation by the NOD2 ligand muramyl dipeptide (MDP), XIAP was recruited to the NOD2 signaling complex by RIPK2. XIAP could next facilitate RIPK2 ubiquitination, which was necessary for downstream activation of NF-κB canonical pathway and of MAP kinases.[97,98] Accordingly, monocytes from XIAP-deficient patients showed severely reduced MDP-induced production of cytokines and chemokines, including TNF-α, IL-8, IL-10, and MCP.[95] Alike NOD2-deficient monocytes, they also displayed impaired MDP-induced clearance of *Salmonella typhimurium* and of adherent-invasive LF82 *E. coli*, a strain associated with Crohn's lesions.[99] Since bacterial autophagy is also defective in macrophages of patients with Niemann–Pick disease C1 (NPC1), a lysosomal lipid storage disorder that can be associated with IBD, Schwerd et al.[99] suggested that it is a common trait driving intestinal inflammation in patients deficient in NOD2, XIAP, or NCP1. A nonexclusive hypothesis implicates excessive activation of inflammasome and cell death. Thus it was shown that, in murine bone marrow-derived dendritic cells stimulated by LPS, XIAP loss can trigger RIPK3- and caspase 8-driven activation of NLRP3, IL-1β secretion, and cell death.[100,101] The mechanism was dependent on the induction of TNF by LPS and involved aberrant ubiquitination of receptor-interacting serine/threonine kinase 1 (RIPK1) in the absence of the RING domain of XIAP.[100] The latter mechanism remains, however, to be demonstrated in humans. Overall, these data point to a key role of XIAP in myeloid cells, in keeping with the curative effect of HSCT on both HLH and intestinal inflammation.[94] Yet, as for NOD2, the precise mechanism by which XIAP deficiency promotes intestinal inflammation is not fully defined. *Xiap*$^{-/-}$ mice showed somewhat increased susceptibility to some but not all bacteria but, alike *nod2*$^{-/-}$ mice, they did not develop spontaneous inflammation (reviewed in[94]). High redundancy between XIAP and related proteins c-IAP1 and c-IAP2 in mice may contribute to the lack or very weak phenotype in this species.

While mouse models have been of little help to delineate the role of the NOD2 pathway in intestinal inflammation, the description of recessive mutations in *TRIM22* as the cause of VEO-IBD supports its importance in humans. TRIM22 is an E3 ubiquitin ligase originally identified as an interferon-inducible protein with antiviral activity. Li et al.[102] showed that TRIM22 associated with NOD2 in epithelial cells stimulated by MDP, leading to NOD2 polyubiquitination and activation of NF-kB and interferon beta signaling. The very severe disease observed in the three affected children stresses the importance of this pathway but, again, the exact scenario leading to intestinal inflammation remains unclear.

## Defects impairing intrinsic regulation of innate immune cells

As highlighted above, chronic intestinal inflammation can result from mutations altering the effector functions of innate immune cells. By impairing microbial clearance, these mutations likely prevent rapid resolution of inflammation. Conversely, mutations that disrupt the regulation of innate cells and enhance their production of proinflammatory cytokines can also induce severe inflammation. They cause heterogeneous disorders, collectively defined as auto-inflammatory diseases (Table 1).

*Defects in intrinsic check-points of the NF-κB cascade*. Patients with *TNFAIP3* haploinsufficiency develop a broad range of early onset immune and inflammatory manifestations including Behçet-like disease with oral and genital ulcers, autoimmune lymphoproliferative syndrome[103] or multiorgan autoimmunity.[104] Approximately 40% patients also display severe inflammatory and ulcerative lesions affecting the entire gastrointestinal tract,[105] supporting the role of TNFAIP3 (TNF α-induced protein 3, also called A20) in human intestinal immunoregulation. This role is corroborated by studies in mice but also by identification of predisposing SNPs in or near *TNFAIP3* in Crohn's and celiac diseases (reviewed in[106]). TNFAIP3/A20 is a potent inhibitor of NF-κB signaling and of cell death that acts by modifying the ubiquitin status of proteins in the NF-κB cascade (reviewed in[106]). Thus, its N-terminal ovarian tumor domain can remove the K63-linked ubiquitin chains that are indispensable to promote NEMO, RIPK1, and TRAF6 recruitment to upstream receptors and to trigger NF-κB activation. Conversely, one of the seven zinc-finger domains, ZnF4, has ligase activity and can add K48-linked polyubiquitin chains, which promote proteasomal degradation of these proteins. A20 can thereby negatively regulate NF-κB activation and, indirectly, that of NLRP3 and STAT1.[106] Recent work in mice further suggests the additional and key role of the ZnF7 domain. This domain, which binds Met1-linked linear polyubiquitin chains, seems indispensable to prevent RIPK1-RIPK3-MLKL-mediated necroptosis of murine macrophages and their release of IL-1β.[107] Observations in mice with tissue-specific inactivation of A20 further suggest that systemic manifestations of inflammation and arthritis result from A20 loss in myeloid cells and activation of the NLRP3 inflammasome.[107] Comparable mechanisms may operate in humans, as peripheral blood mononuclear cells (PBMCs) from A20-deficient patients showed constitutive activation of NLRP3 and increased secretion of IL-1β and IL-18. Moreover, one patient responded to IL-1 blockade by Anakinra.[108] Of note, in mice, selective deletion of A20 in intestinal epithelial cells increased markedly epithelial apoptosis in response to DSS and TNF in mice. It is therefore not excluded that A20 loss in intestinal epithelial cells may also contribute to the severe ulcerative enteritis observed in some patients (reviewed in[106]).

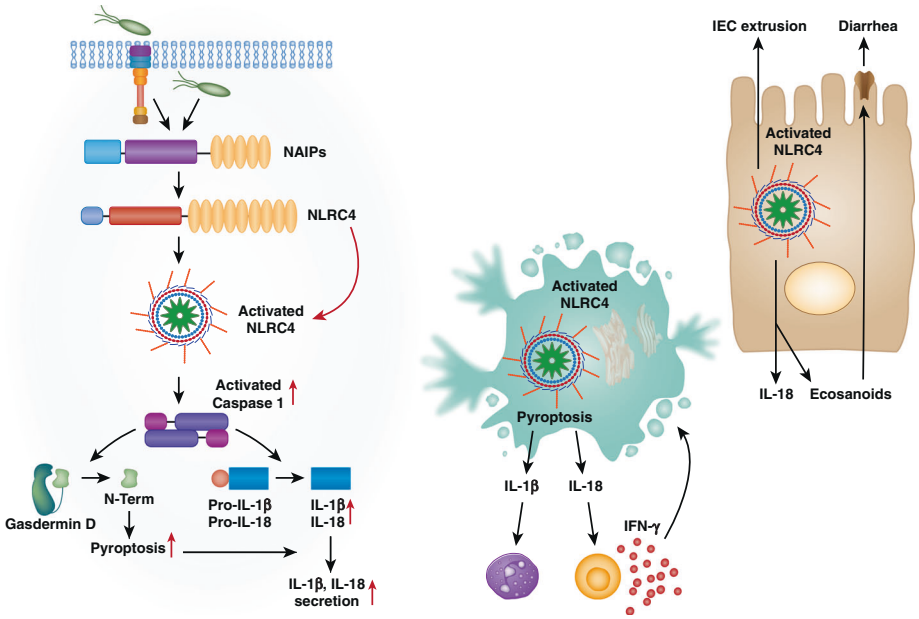


**Fig. 5 Mechanism of intestinal inflammation in activating NLRC4 variants.** Mutations in NLRC4 autoinhibitory domain result in spontaneous activation of NLRC4 inflammasome in myeloid and epithelial cells (independently of binding of apoptosis inhibitory proteins NAIPs and microbe-derived signals). NLRC4 activation in macrophages triggers pyroptosis and secretion of mature IL-1β and IL-18. IL-18-induced production of IFNγ can cause life-threatening macrophage activating syndrome. NLRC4 activation in epithelial cells not only triggers IL-18 production but also eicosanoid production, resulting in diarrhea and epithelial cells (IEC) extrusion.

Along with A20 deficiency, a severe neonatal disease combining systemic inflammation, neutrophilic dermatitis, and diarrhea has been reported in some rare patients harboring biallelic hypomorphic mutations in OTULIN. This molecule is a deubiquitinase, which specifically removes the Met1-linked linear polyubiquitin chains added by linear ubiquitin chain assembly.[109,110] In contrast to A20 deficiency, but in keeping with data in mice pointing to a key role of OTULIN in the negative feedback of NF-κB-dependent production of inflammatory cytokines and notably of TNF in myeloid cells, the disease was controlled by anti-TNF treatment (infliximab) but not by anti-IL-1β.[110]

*Defects in intrinsic check-points of inflammasomes*

NLR family CARD domain-containing protein 4 (NLRC4) GOF mutations and intestinal inflammation: As discussed above, excessive production of IL-1β by macrophages might participate in intestinal inflammation. In this regard, it is somewhat unexpected that patients with GOF mutations in the NLRP3 inflammasome, which lead to excessive release of IL-1β by myeloid cells, develop severe inflammation in many organs but not in the intestine (reviewed in[111]). The need for strict regulation of the inflammasome to maintain intestinal homeostasis is, however, underscored by identification of GOF mutations in *NLRC4*. Thus, patients with *NLCR4* GOF mutations develop a severe disease combining neonatal enterocolitis and macrophage activation syndrome (MAS).[112,113] Inflammation can extend to lungs, central nervous system, and joints. Activation of the NLRC4 inflammasome is normally triggered by flagellin or by the type III secretion system needle. These ligands do not bind directly NLRC4 but interact with the apoptosis inhibitory proteins (NAIP), which can then bind the leucin-rich-repeat domain of NLRC4 (reviewed in[114]). NAIP binding induces a conformational change in the autoinhibitory NOD domain of NLRC4, which enables oligomerization and activation of several NLRC4 molecules within a large wheel-shaped structure (Fig. 5). Following assembly of this complex, NLRC4 caspase recruitment domains (CARD) cluster and recruit procaspase 1 either directly or via the apoptosis-associated speck-like (ASC) molecules.[114] NLRC4 is expressed both in myeloid cells (macrophages and neutrophils) and in IEC. In myeloid cells, inflammasome formation initiates caspase 1-dependent cleavage of pro-IL-1β and pro-IL-18 that accumulate in response to TLR signals. In parallel, caspase 1 cleaves Gasdermin D, inducing pyroptosis and the subsequent extracellular release of active IL-1β and Il-18[114] (Fig. 5). Accordingly, macrophages of patients carrying mutations in NLRC4 autoinhibitory domain can release large amounts of both cytokines in response to TLR activation (signal 1) in the absence of signal 2 by NLRC4 ligands.[112,113] IL-18 being a potent inducer of IFNγ, it is likely the trigger for MAS that is not observed in patients with GOF mutations in *NLRP3*, which preferentially induce IL-1β overproduction.[111] Accordingly, whole blood transcriptional analysis in patients with NLRC4-MAS showed a strongly enriched IFNγ signature. While abnormal activation of NLRC4 in patients' myeloid cells is undoubtedly the cause of MAS, its role in gut pathology is less clear and data in in mice suggest an additional role of NLRC4 in IEC. Indeed, NLRC4 activation in mouse IEC was shown to simultaneously stimulate lytic death and expulsion of IEC, IL-18 production and local release of eicosanoids that induced intraluminal fluid loss.[115] This mechanism protected mice against intestinal infection by *Salmonella* but, conversely, could induce severe intestinal pathology when inappropriately activated. Since intestinal symptoms subsided in *NLRC4*-mutated patients surviving the 1st year of life, a triggering role of early gut colonization was suggested.[116] Importantly, the central role of IL-18 in MAS and enterocolitis resulting from *NLRC4* GOF mutations was demonstrated by the spectacular therapeutic efficacy of IL-18 blockade in a few-month-old baby girl, who had only been marginally improved by aggressive immunosuppression including IL-1 and TNF blockade.[113]

Mevalonate kinase deficiency (MVKD) and intestinal inflammation: MVKD manifests by recurrent fever, abdominal pain with diarrhea and vomiting, arthralgias, and mucocutaneous lesions. Mutations can be mono- or biallelic and leave variable residual enzymatic activity. As a result, disease severity varies broadly from a mild form called hyperimmunoglobulinemia D and periodic fever

syndrome to severe mevalonic aciduria that can be associated with dysmorphisms and delayed psychomotor development (reviewed in[117]). Repeated attacks of peritonitis can induce adhesions and lead to intestinal obstruction. Some patients also develop colitis. Very severe neonatal colitis or ileocolitis have notably been reported in mevalonic aciduria.[118] Symptoms have been linked to hyperactivation of monocytes and to their excessive production of cytokines, notably IL-1β, TNF, and IL-6. Accordingly, patients are improved, although variably, by blocking these cytokines. Of note, anti-IL-1β therapy, which is reported to be efficient in only 35% of MVK-deficient patients cured the two cases of VEO-IBD.[118] How MVK deficiency leads to overproduction of inflammatory cytokines is not entirely clear. One attractive hypothesis points to defective production of geranyl–geranyl phosphoresidues that are major metabolites of the mevalonate pathway downstream of the phosphorylation of mevalonate catalyzed by MVK (Fig. 4b). Geranyl-geranylation of the RhoGTPase KRas was shown to be necessary for TLR-induced activation of the PI3K catalytic subunit p110d (PIK3cδ), which, in turn, inhibits TLR-mediated inflammatory signals through activation of AKT1 and mechanistic target of Rapamycin (mTOR).[119] Accordingly, mouse bone marrow-derived macrophages (BMDCs) lacking Ppgt1b, the enzyme indispensable for geranyl-geranylation of Kras, or lacking PIK3cδ produced markedly increased amounts of IL-1β, IL-6, IL-12, and TNF but much less IL-10 in response to stimulation by LPS.[119] Importantly, overproduction of IL-1β in *Ppgt1b*$^{-/-}$ BMDCs was shown to depend on the PYRIN inflammasome as IL-1β release subsided when expression of PYRIN, of caspase 1, and ASC but not of NLRP3 or AIM2 was inhibited or deficient in *Ppgt1b*$^{-/-}$ BMDC (Fig. 4b). A similar link between defective geranyl-geranylation of RhoA and PYRIN-dependent excessive production of IL-1β was described in human PBMC treated by Simvastatin to inactivate the mevalonate kinase pathway.[120] Indeed, RhoA was necessary to activate the serine-threonine kinases PKN1 and PKN2, which, in turn, phosphorylated PYRIN, allowing its binding to the 14-3-3 protein. This regulatory protein prevented PYRIN binding to ASC and thereby avoided activation of the PYRIN inflammasome at steady state. Inactivation of RhoA by bacterial toxins or as a consequence of defective geranyl-geranylation in MVK deficiency released the active form of PYRIN, triggering the oligomerization of ASC and the activation of caspase 1[120] (Fig. 4b). This scenario also allows to establish a mechanistic connection between MVKD and Familial Mediterranean fever. This auto-inflammatory syndrome, which is highly prevalent in populations originating from the Eastern Mediterranean, is due to bi- or more rarely monoallelic mutations in the *MEFV* gene that encodes PYRIN. Most mutations, by impairing PYRIN interactions with PKN1, prevent the phosphorylation that is necessary for binding 14-3-3 and avoid inflammasome activation.[120] Patients develop recurrent attacks of fever and very painful aseptic serositis, notably peritonitis. Yet, despite a few reports, bona fide IBD seem rare,[121] raising again questions on why only a subset of diseases associated with excessive production of IL-1β by myeloid cells lead to intestinal inflammation. Excessive activation of the inflammasome and production of IL-1β as a cause of intestinal inflammation have, however, been again suggested in two recently described monogenic diseases that impair RIPK1 and caspase 8.

*Defects in intrinsic regulation of apoptosis or necroptosis of innate immune cells*

Mutations in receptor-interacting serine/threonine kinase 1 (RIPK1): Biallelic LOF mutations in *RIPK1* were recently identified in patients displaying a variable combination of immunodeficiency, early onset IBD affecting both the upper and lower gastrointestinal tract and severe polyarthritis.[74,122] RIPK1 plays an important role downstream of the TNF receptor and of TLRs by acting both as a docking protein necessary to activate NF-κB and MAP-kinase signaling, but also as a kinase implicated in caspase 8-mediated apoptosis or RIPK3-MLKL dependent necroptosis. Defective NF-κB and MAP-kinase signaling likely explains increased susceptibility to infections but the mechanism of intestinal inflammation is not fully elucidated. *Ripk1*$^{-/-}$ mice as well as mice selectively depleted in Ripk1 in IEC showed severe intestinal lesions and extensive IEC death that were ascribed to loss of the scaffolding function of RIPK1 and reduced stability of the survival proteins TRAF2 and c-IAP1.[77] In contrast, there is no evidence of extensive epithelial cell death in patients and intestinal inflammation has rather been related to the abnormal response of macrophages to LPS with both increased activation of NLRP3 inflammasome and increased necroptosis that ultimately result in increased production of IL-1β.[74,122] Resolution of IBD and arthritis after HSCT in one patient supports a prominent contribution of hematopoietic cells.[122] The complex role of RIPK1 in regulating cell death as well as the difficulty to translate data from animal models to humans are further highlighted by observations in patients carrying mutations that abolish RIPK1 binding to caspase 8. Thus, cleavage of RIPK1 by caspase 8 was shown to curb RIPK1 kinase activity and thereby to prevent RIPK3-MLKL dependent necroptosis.[123] Accordingly, mutations that abolished RIPK1 cleavage by caspase 8 resulted in GOF with not only increased spontaneous and LPS-induced production of inflammatory cytokines but also enhanced apoptosis and necroptosis of PBMCs in response to TNF.[124,125] Again and in contrast with mice carrying a comparable mutation,[123] there was no evidence of excessive IEC death in patients, and they developed severe systemic inflammation but, curiously, no intestinal disease.[124,125] Similar paradoxical observations have been made in caspase 8 deficiency.

Caspase 8 deficiency is embryonically lethal in mice. This is not the case in humans, probably due to redundancy with caspase 10, which does not exist in mice.[126] Caspase 8 deficiency was initially described in patients with multiorgan lymphocyte infiltration and severe immunodeficiency (reviewed in[70]). These symptoms were ascribed to the role of caspase 8 in the proapoptotic cascade downstream of FAS and to the facilitating role of caspase 8 in NF-kB activation downstream of B- and T-cell receptors.[70] Recently, *CASP8* LOF mutations were also identified in patients combining immunodeficiency and very severe colitis with perianal lesions.[75] As in RIPK1 deficiency, how caspase 8 deficiency causes intestinal inflammation is not clear. In mice, as alluded above, caspase 8 inhibited TNF-induced cell death through the cleavage of RIPK1,[123] and its selective deletion in IEC led to TNF-induced epithelial necroptosis and spontaneous ileitis.[76] Yet, intestinal organoids derived from caspase 8-deficient patients were insensitive to TNF-induced cell death and patients were not improved by anti-TNF treatment.[75] In contrast, activation by LPS of human macrophages deleted in caspase 8 induced, as in RIPK1-deficient macrophages, necroptosis and, as a result, activation of NLRP3 and secretion of IL-1β,[75] suggesting again a possible role of IL-1β in colitis. There is, however, no evidence as yet that IL-1β blockade might improve colitis in RIPK1- or caspase 8-deficient patients.

## Defects in extrinsic regulation of intestinal immune cells: IL-10 and TGF-β

Defects discussed above demonstrate the importance of intrinsic regulation of signaling cascades in innate immune cells to preserve intestinal homeostasis in humans. Data below illustrate the complementary key role of extrinsic regulation by two immunoregulatory cytokines, IL-10 and TGF-β (Table 1).

*IL-10 and regulation of intestinal innate immune cells*. The crucial role of IL-10 in human colonic homeostasis has been definitively established with the identification of LOF mutations in *IL10RA*, *IL10RB*, and *IL10*[127,128]. In the over 130 reported cases (almost all affecting the receptor), all children developed within the first

weeks or months of life severe colitis refractory to medical treatment with perianal lesions and with granulomas on histological sections.[129–131] Strikingly and despite many studies in mice suggesting a regulatory role of IL-10 on a broad spectrum of immune responses, patients displayed very few other symptoms except for frequent skin folliculitis and, rarely, arthritis. Of note, clinical presentation was comparable in patients with mutations in IL-10RA, the IL-10-specific receptor chain, and in patients with mutations in IL-10RB, which is necessary for signaling downstream IL-10 but also IL-22, IL-26, and interferon-λ.[129–131] Strikingly, HSCT, the only treatment to date,[129,132] can cure all symptoms. Overall, these observations indicate that, in humans, colonic homeostasis depends on IL-10 signaling in cells of hematopoietic origin. The therapeutic efficacy of HSCT pleads against a major role of IL-22 signaling in IEC, a result that is surprising given evidence of the importance of IL-22 in stimulating epithelial defenses in mice.[133] The lack of increased susceptibility to gastrointestinal viral infections in *IL10RB*-deficient patients is also unexpected given evidence of the protective role of interferon-λ in mouse models.[134] It is therefore likely that, in humans, type I interferons enable mucosal protection in the absence of interferon-λ signals. Importantly, HSCT also prevented the risk of developing B-cell lymphomas, an as yet poorly understood complication of IL-10R deficiency in humans.[135]

The central role of IL-10 in the regulation of intestinal immune responses to the microbiota was first highlighted in *il10*$^{-/-}$ mice.[136] Thus the latter mice developed severe intestinal inflammation that was attenuated in specific pathogen-free mice[136] and abolished in germ-free mice.[137] Elegant studies using conditional inactivation of IL-10 or of its receptor in various cell types next showed that IL-10 production by T cells, and notably by regulatory T cells, was indispensable to prevent the onset of spontaneous colitis,[138,139] while, conversely, colonic macrophages were the main target of the anti-inflammatory role of IL-10[140–142]. In keeping with these findings, IL-10 exerts a potent inhibitory effect on the inflammatory response of human peripheral monocytes to LPS and loss of this inhibitory effect is routinely used to screen for IL-10R deficiency.[131,143] The mechanisms of the anti-inflammatory action of IL-10 remain, however, incompletely understood. Signaling is initiated by binding of IL-10 to IL-10RA, which can then recruit IL-10RB, resulting in the formation of heterotetramers, activation of the kinases Tyk2 and JAK1 (that are constitutively associated with IL-10RA and IL-10RB, respectively), and ultimately recruitment and activation of STAT3 (reviewed in[129]) (Fig. 4c). Yet, STAT3 is downstream of many cytokine receptors, including receptors for inflammatory cytokines such as IL-6. Moreover, LOF mutations in Tyk2 and STAT3 have a clinical outcome very different of IL-10R mutations with notably no colonic inflammation (reviewed in[144,145]), while, conversely, STAT3 GOF mutations can result in severe intestinal inflammation (see below). IL-10 was also shown to suppress LPS-induced glycolytic shift in murine macrophages by blocking translocation of the GLUT1 transporter to the surface membrane as well as mTORC1 activation (Fig. 4c). By blocking mTORC1, IL-10 stimulated autophagy and thus allowed elimination of dysfunctional mitochondria producing excessive amounts of ROS[146] but also likely degradation of NLRP3, NLRC4, and ASC as discussed above.[89] As a result, IL-10-deficient murine macrophages activated by LPS displayed excessive activation of inflammasome and IL-1β secretion[146] (Fig. 4c). An inhibitory role of IL-10 on NLRP3 inflammasome activation and IL-1β production was also observed in humans and, strikingly, IL-1 receptor antagonist anakinra could improve intestinal inflammation in two patients with IL-10R deficiency.[147] Mechanistically, mTOR inactivation by IL-10 was shown to involve STAT3-dependent induction of the mTOR inhibitor DDIT4[146] (Fig. 4c). It remains, however, unknown whether DDIT4 is uniquely induced by IL-10 downstream STAT3 and, if so, why. A very recent study using single cell transcriptomics further stresses the importance of IL-10 in regulating IL-1 production by monocytes by showing a paracrine effect of IL-1 (α or β) on IL-23 production. Accordingly, monocytes from IL-10R-deficient patients incubated with IL-10 or control monocytes treated by anti-IL-10R antibody produced increased amount of IL-23 after LPS stimulation (Fig. 4c). The latter cells also showed high expression of IL-1α or β, IL-6, TNF, and oncostatin M.[148] Of note, other subsets of monocytes stimulated by LPS in the presence of anti-IL-10R antibody had increased expression of type I IFN-responsive genes, of genes involved in superoxide generation or antigen presentation, highlighting a very broad inhibitory effect of IL-10 on monocyte functions beyond IL-1 regulation.[148]

Interestingly, recent work suggests that the colitis, which can develop in patients with mutations in the Wiskott–Aldrich protein (WASP), may be related to the impairment of IL-10 signaling in colonic macrophages. WASP, a protein implicated in the regulation of actin skeleton, was shown to be necessary for optimal phosphorylation of STAT3 upon IL-10 stimulation. A comparable role was, however, not formally demonstrated in WASP-deficient human monocytes, although they displayed enhanced proinflammatory characteristics alike colonic macrophages in *wasp*$^{-/-}$ mice.[149] Finally, dysregulation of the IL-10 pathway may perhaps also participate in common forms of IBD. Thus, IBD association with polymorphisms in the *IL10* gene, although controversial, is comforted by a recent metanalysis.[150]

*TGF-β and regulation of intestinal innate and adaptive immune cells.* TGF-β1–3 belong to a large family of 32 cytokines with key roles in development. While the three TGF-β isoforms share strong sequence homology and the two chains and of their receptor, they do not share redundant functions.[151,152] Thus, only mice inactivated for TGF-β1 develop rapidly lethal multiorgan inflammation, pointing to the distinctive crucial role of TGF-β1 in immune homeostasis.[153] Recent reviews have precisely described the broad distribution of cells producing and responding to TGFβs, the complex mechanisms of activation of TGF-β from an inactive proprotein, and the multiple mechanisms by which TGF-β1 participates in immune homeostasis.[151,152,154] Briefly, TGF-β1 is cleaved during its traffic within the Golgi apparatus but remains bound to the latency-associated peptide (LAP) and is released from cells as an inactive homodimer, which is deposited in the extracellular matrix (ECM). Upon interaction of LAP with thrombospondin in ECM or with the $\alpha_V$ integrins expressed at the surface of a broad range of cells, active TGF-β1 is released and binds TGF-β type II receptor (TGF-βR2), inducing the recruitment and phosphorylation of TGF-β type I receptor (TGF-βR1) and its kinase activity. This leads either to phosphorylation of SMAD2 and SMAD3 and activation of the canonical SMAD pathway or, alternatively, to activation of PI3K, RhoGTPases, or various MAPK.[151,152,154] The SMAD pathway plays a major role in the immunoregulatory effects of TGF-β.[152] Thus, following their phosphorylation, SMAD2 and SMAD3 form a trimer with either SMAD4 or TIF1γ and translocate into the nucleus where they bind to regulatory regions and interact, in a context-dependent manner, with multiple other regulatory proteins to influence the transcription of genes involved in immunoregulation.[151,152] Data in mice indicate that TGF-β can thereby directly inhibit CD4$^+$ TH1, TH2 responses as well as cytotoxic CD8$^+$ T-cell responses, promote differentiation of regulatory T cells (Tregs), inhibit antigen presentation by dendritic cells and NF-kB activation and production of inflammatory cytokines in macrophages (reviewed in[151,155]). A substantial number of patients with heterozygous LOF mutations in *TGFB1*, *TGFB2*, *TGFB3*, *TGFBR1*, *TGFBR2* and in *SMAD3* have been identified. In keeping with the key role of TGFβ-2 and -3 in development, patients display skeleton and connective tissue disorders and, notably, severe cardiovascular defects.[156,157] In addition, a substantial proportion of patients with heterozygous TGF-βR1 or R2 LOF mutations were reported to develop allergic

symptoms, including food allergy (>50%), asthma (45%), eczema (38%), eosinophilic gastrointestinal diseases with increased peripheral eosinophils, elevated serum IgE and plasma levels of IL-5 and IL-13. Paradoxically, the frequency of peripheral blood Tregs was increased and their ability to suppress T-cell proliferation was normal. Moreover, they produced increased amounts of IL-13 and IL-17 and in vitro CD3 stimulation of naïve CD4+ T cells led to generation of forkhead box protein 3 (FOXP3) T cells producing IL-13.[158] Increased incidence of IBD, including four cases of severe VEO-colitis was also reported in patients with TGF-βR1 or R2 LOF mutations, associated in one child with thyroid autoimmunity.[159,160] These findings may appear in keeping with data in mice showing that TGFβ1 can suppress TH1 and TH2 responses.[151,158] Strikingly, however, and despite clear demonstration that all mutations were LOF, most if not all symptoms have been associated or even ascribed to a paradoxical increase in TGFβ signaling, notably attested by increased translocation of phosphoSMAD2/3 in affected tissues and in peripheral T cells stimulated by TGF-β1.[156,158] How to reconcile such increase with the observed immune symptoms remains uneasy. Easier to explain, strongly reduced TGF-β1 signaling has been recently reported in three children with biallelic mutations in *TGFβ1* that impaired its release or stability. All three developed severe ulcerative pancolitis within the first months of life,[161] but also a very severe neurological disease of unknown mechanism. Decreased numbers of Tregs were observed in the only child who was tested together with eosinophilic oesophagitis, increased serum concentration of IgG and IgE. The children also displayed recurrent infections and decreased TH1 and TH17 cell responses but the role of heavy immunosuppression was not excluded.[161] Of note, polymorphisms in *SMAD3* have been associated with increased risk to develop IBD.[162] Overall, these data support an important role of TGF-β1 in restraining colonic inflammation but also adverse TH2 responses to food antigens. Cellular targets and signaling relays affected in humans by TGF-β dysregulation remain, however, not fully elucidated.

## Intrinsic defects impairing activation and regulation of adaptive immune cells

A considerable and constantly expanding number of gene defects are known to impair development and or function(s) of adaptive immune cells.[54] We will discuss successively how B- and T-cell defects illustrate the respective contributions of antibodies and T cells to intestinal homeostasis in humans (Table 1).

*Impairment of intestinal defenses and immunoregulation in B-cell defects*. A broad spectrum of B-cell defects has been described in humans, which affect variably B-cell functions and antibody production. Attesting the importance of antibody in protection of mucosal surfaces, they all confer risk of recurrent sinopulmonary and intestinal infections of bacterial and viral origins. As discussed below, this risk is, however, variable, revealing redundancy in the protective role of the different isotypes, notably between IgA and IgM. Intestinal inflammation and autoimmune disorders are severe complications, the frequency of which varies depending on B-cell defects and on impairment or not of other cell subsets, notably T cells. Three main conditions are discussed below.

X-linked agammaglobulinemia (XLA): XLA is caused by LOF mutations in *Btk*, which encodes the Bruton tyrosine kinase on the X chromosome. This kinase, that is indispensable for B-cell development, differentiation, activation, proliferation, and survival, is activated in response to B-cell receptor crosslinking and, in turn, activates PLC- γ2 and $Ca^{++}$ mobilization (reviewed in[163]). BTK deficiency therefore results in profound decrease or absence of peripheral B cells and lack or severe decrease in all immunoglobulin isotypes.[54] Thanks to intravenous supplementation in immunoglobulins, frequency of life-threatening systemic infections by encapsulated bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*), as well as of chronic meningoencephalitis induced by enteroviruses, has markedly decreased. Yet, over 50% of the patients still develop recurrent sinopulmonary infections, exposing to the risk of bronchiectasis, and 35% display gastrointestinal complications, mainly related to infectious agents (including *Giardia lamblia*, rotavirus, adenovirus, *Campylobacter jejuni*, and *Clostridium difficile*), overall reflecting the fact that intravenous immunoglobulins, chiefly IgG, cannot easily compensate for the anti-infectious functions of IgA and IgM at mucosal surfaces.[164] Although much rarer than in common variable immunodeficiency (CVID, see below), Crohn-like disease has been reported in 3–4% of patients. Curiously, however, this complication concerns mainly patients with XLA hypomorphic variants.[165] The underlying mechanism thus remains unclear and may not depend (exclusively) on the lack of immunoglobulins. Indeed, BTK is expressed in myeloid cells where it can negatively regulate TLR signaling, but also modulate ROS induction by neutrophils (reviewed in[163]).

Selective IgA deficiency (SIgAD): SIgAD is defined by serum IgA < 0.07 mg/mL after 4 years of age but normal serum IgG and IgM. With an incidence of about 1:600 in the western world, it is the most frequent immunodeficiency. In contrast to XLA, genetic origin of SIgAD is unknown and likely complex.[166,167] Many cases are asymptomatic or with mild symptoms, suggesting redundancy with IgM for mucosal protection. Accordingly, IgM fecal concentrations are increased in SIgAD and fecal bacteria were found to be coated by IgM instead of IgA, likely explaining why microbiota composition is much less altered than in patients with CVID, who lack both IgA and IgM[168,169] Yet redundancy is incomplete as the risk of developing sinopulmonary and intestinal infections is increased more than threefold compared to the general population.[170] Moreover, SIgAD individuals were shown to display mild dysbiosis with some expansion of proteobacteria when compared to healthy donors. They also had evidence of systemic immune activation with modest but significant increase in serum concentrations of IL-6, IL-17, and IL-10 and soluble CD14, as well as increase in circulating CD4+ TH17 cells[168] suggesting that, in absence of IgA, some bacterial translocation can occur, a hypothesis further supported by the increase in serum IgG directed against commensal bacteria.[171] Whether these changes may contribute to other complications of SIgAD is, however, unknown. While the risk of allergy remains disputed (reviewed in[166]), SIgAD is indeed associated with increased prevalence of autoimmune diseases with up to 25–30% concerned patients in some cohorts. Moreover, many patients even without symptoms develop autoantibodies.[167] The exact mechanism remains uncertain but the increased prevalence of autoimmunity in first degree relatives without IgA deficiency as well as the overlap between HLA loci predisposing to SIgAD and to celiac disease and type I diabetes led to suggest common but non causal genetic susceptibility.[167] Of note, SIgAD can precede or be associated in the same family with CVID, pointing to some overlap between the mechanisms underlying these two conditions.

Common variable immunodeficiency (CVID): CVID is defined by markedly reduced serum IgG, decreased IgM or/and IgA, and poor antibody responses to vaccines in the absence of secondary cause of hypogammaglobulinemia. Patients have low or normal B-cell counts but always reduced numbers of isotype-switched $CD27^+$ memory B cells, reflecting impaired B-cell differentiation in antibody-secreting cells.[172] In contrast to XLA, CVID is a highly heterogeneous condition with very variable clinical presentation and severity and considerable immunological and genetic diversity.[173,174] Most cases manifest during the third and fourth decades and are thought to be of multifactorial origin. Yet, recent use of next generation sequencing suggests

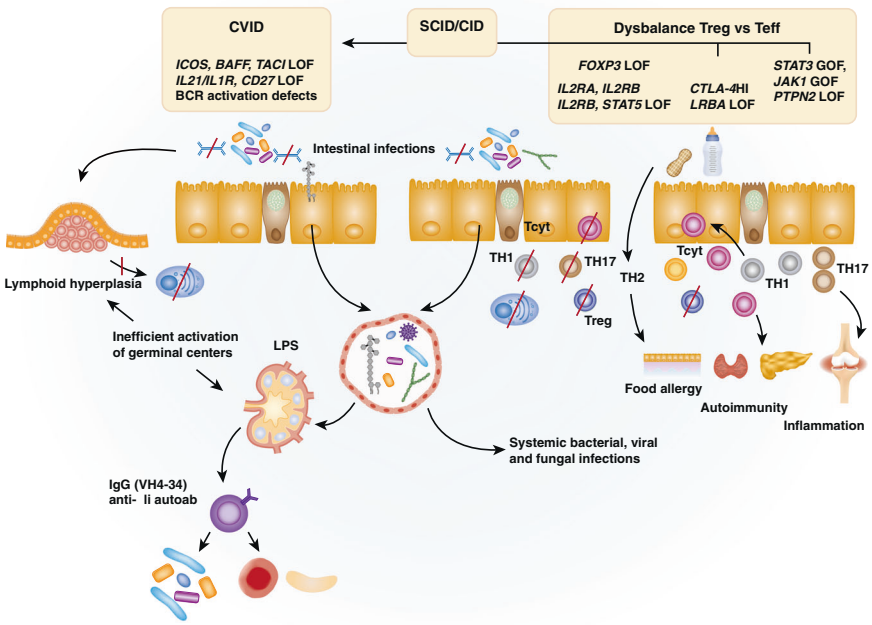


**Fig. 6 Summary of adaptive immune defects that impair intestinal homeostasis.** In common variable immunodeficiencies (CVID), impaired production of both IgA and IgM promotes intestinal infections but also dysbiosis and increased translocation of LPS and microbes. The gut immune barrier is further impaired in patients with severe combined immunodeficiencies (SCID) or combined immunodeficiencies (CID), with severe profound T-cell defects. Intravenous supplementation in immunoglobulins reduces the risk of systemic bacterial and viral infections of intestinal origin. Yet, in CVID, translocation of LPS can induce inefficient stimulation of germinal centers, leading to lymphoid hyperplasia and production of IgG with an unmutated VH4-34 heavy chain that cross-react with commensal bacteria and the I/i carbohydrate autoantigen expressed by erythrocytes and platelets.[177,178] Defects that selectively lessen numbers or function of Tregs or enhance effector T-cell activation result in intestinal autoimmunity and lymphocytic inflammation, respectively. Food allergy is frequent in Treg deficiencies. Several gene defects affecting the Treg to effector T-cell balance can lead to CVID by mechanisms that are not fully elucidated (see text).

that 8–30% of CVID, depending on the cohorts, may be favored by specific gene defects. CVID can notably develop in patients carrying mutations in genes necessary for germinal center formation (ICOS: induced T-cell costimulator), B-cell differentiation and survival (BAFFR: B-cell activating factor receptor; TACI: transmembrane activator; and CAML: calcium-modulating cyclophilin ligand interactor, CD27, IL-21, and IL-21 receptor), genes encoding BCR costimulatory molecules (CD19, CD20,CD21, CD81), or signaling molecules downstream BCR (and TCR) activation, with notably LOF mutations in PKCδ, PLCγ2, NFκB-1,-2, and GOF mutations in the PI3Kδ pathway (reviewed in[172,173,175]). CVID can also be the consequence of mutations affecting predominantly T-cell functions, and notably T-cell help, as discussed below and more generally reviewed in.[172,173,176] Whatever the genetic origin, the most common clinical consequence of CVID is an increased susceptibility to respiratory bacterial and viral infections, which is partially prevented by intravenous immunoglobulin supplementation.[173] A substantial number of patients also develop autoimmune disorders, notably antibody-mediated cytopenia (20–30%) and/or digestive manifestations (50%) such as chronic diarrhea and malnutrition, gastrointestinal inflammation and lymphoid hyperplasia (Fig. 6). The risk of lymphoproliferative disorders and solid tumors is also increased by 30- and 5-folds, respectively (reviewed in[174]). The mechanisms of these manifestations are complex and likely influenced by the nature of the underlying deficiency, although most reports do not distinguish clearly the case of patients with or without identified genetic cause. Overall, autoimmunity seems more prevalent in patients with severe IgA deficiency,[177,178] while severe digestive symptoms are associated with profound depletion in intestinal plasma cells.[179]

Interestingly, recent work has linked autoimmune cytopenia in IgA-deficient patients with increased endotoxemia, which may stimulate excessive activation of follicular helper T cells, inefficient activation of germinal centers, and production by $IgG^{+}$ B cells ($CD19^{hi}$ $CD21^{neg/lo}$) of antibodies encoding an unmutated VH4-34 heavy chain that cross-reacts with commensal bacteria and the I/i carbohydrate antigen expressed by erythrocytes and platelets[177,178] (Fig. 6). As in XLA, gastrointestinal symptoms can be caused by pathogens such as *Helicobacter pylori*, *Giardia lamblia*, *Campylobacter jejuni*, *Salmonella*, *Cryptosporidium*, or *Norovirus* notably.[172,179] Yet, in many instances, no specific pathogen is detected and the characteristics of intestinal inflammation point out to immune dysregulation, with notably Biermer-like gastritis, celiac-like duodenitis (generally resistant to gluten free-diet), graft-versus-host disease-like, or Crohn-like lesions in the distal intestine. Intestinal inflammation, notably in the distal intestine may be favored by the dysbiosis associated with CVID.[168,169] It likely also reveals impairment of T-cell immunoregulation, as exemplified below in the cases of cytotoxic T lymphocyte antigen 4 (*CTLA-4*) and LPS-responsive beige-like anchor protein (*LRBA*) LOF mutations or *STAT3* GOF mutations, which can lead to CVID. Accordingly, immunoglobulin supplementation is generally inefficient to treat intestinal inflammation, while patients can be substantially improved by oral corticoids[179] or by treatments targeting specifically the gene defect (see below).

*Impairment of intestinal defenses and immunoregulation in T-cell defects.* A considerable number of T-cell defects described so far may affect intestinal defense and or intestinal immunoregulation. After summarizing the consequences of severe global T-cell

defects, we will focus on selected gene defects, which impair T-cell regulatory functions and/or enhance effector T-cell functions. These defects are frequently associated with severe intestinal inflammation that manifests predominantly in the small intestine as autoimmune enteropathy.

Severe global T-cell deficiencies: Severe combined immunodeficiencies (SCID) are defined by profound decrease or absence of endogenous T lymphocytes. Early onset intractable diarrhea is constant and completes clinical presentation dominated by life-threatening infections, notably pulmonary infections or infections originating from the intestine.[180] Diarrhea is common in gene defects affecting both T cells and innate lymphoid cells as in X-linked SCID due to LOF mutations in *IL2RG* encoding the $\gamma_c$ chain, in defects that affect V-(D)-J recombination in T and B cells, e.g., mutations in recombination-activating protein 1 (RAG1) and 2 (RAG2) or exclusively T-cell differentiation as in *IL7RA* mutations,[180] overall stressing the indispensable and central role of T cells to build an efficient intestinal barrier. Besides typical SCID, a broad spectrum of genetic defects impairing T-cell differentiation or function can not only increase susceptibility to infections but also result in autoimmunity and inflammation which often affect the gut. These are often divided in "atypical SCID" caused by hypomorphic mutations in SCID genes and "combined immunodeficiencies" reflecting the fact that, whether or not associated with intrinsic B-cell defects, B-cell functions are impaired by the lack of T-cell help. They have been exhaustively reviewed in.[180,181] One interesting situation concerns hypomorphic RAG defects, in which a small number of T and B cells can undergo V-(D)-J recombination, while thymic differentiation of Tregs is severely impaired. The few rearranged T cells undergo oligoclonal expansion, resulting in massive infiltration of multiple organs including the small and large intestines, while abnormal B-cell differentiation prevents development of IgA plasma cells and simultaneously licenses expansion of autoreactive B cells producing IgG and IgE (Ommen syndrome).[182] Reminiscent of observations in CVID, inflammation seems to be driven by microbiota. Thus, RAG hypomorphic mice engineered to reproduce the human mutations, showed severe dysbiosis with reduced bacterial diversity, increased number of mucosa-adherent proteobacteria, and reduced bacterial IgA coating.[183] Moreover, both intestinal and systemic inflammation were improved by oral antibiotics, which decreased numbers of intestinal and circulating TH1 and TH17 cells and reduced translocation of microbiota-derived products,[183] overall underscoring importance of fully functional and tightly regulated adaptive immunity to cope with microbiota and maintain intestinal and systemic homeostasis (Fig. 6).

Defects impairing development and functions of FOXP3 regulatory T cells (Tregs): The seminal description of the human immune dysregulation polyendocrinopathy and enteropathy X-linked syndrome (IPEX) syndrome was made in 1982 in a family comprising, over three generations, 17 boys diversely affected by diarrhea, autoimmune diseases, eczema-like skin lesions, and increased sensitivity to infections[184] (Fig. 6). Confirming the X-linked inheritance suggested by this pedigree, IPEX syndrome was mapped to the *Xp11.23–Xq13.3* region,[185–187] that is syntenic to the murine Xp11.23–p11.22 region containing the Scurfy locus. LOF mutations were then almost simultaneously identified in mice[188] and in humans in a new member of the forkhead/winged-helix family of transcriptional regulators that was first named JM2[185] and rebaptized FOXP3.[189,190] FOXP3 was then rapidly shown to be a canonical marker for $CD4^+CD25^+$ Tregs and a master regulator of their suppressive function.[191–193] $CD4^+CD25^+$ Tregs were first described in 1985 by S. Sakaguchi for their role in maintaining immunological self-tolerance in the periphery. Thus, neonatally thymectomized mice, which lacked thymus-derived Tregs, developed gastritis, thyroiditis, oophoritis, and orchitis together with organ-specific autoantibodies.[194] Selective depletion of FoxP3 Tregs at the adult age confirmed their key role in restraining self-reactivity.[195] A subset of Tregs was also shown to develop from naive $CD4^+$ T cells in the periphery presumably in response to environmental antigens. Conversion of naive T cells with a monoclonal T-cell receptor specific for ovalbumin into functional Foxp3+ Tregs was notably observed in mesenteric lymph nodes after oral exposure to this antigen.[196] In addition, Tregs displaying T-cell receptors with reactivity against microbiota antigens could expand in the colonic mucosa of mice colonized by the relevant bacteria,[197] and microbiota was shown to induce a specific subset of $ROR\gamma t^+$ Tregs in the colon.[198,199] The latter data, together with recurrent demonstration that Tregs prevent the colitis induced by transfer of naive $CD4^+$ T cells into lymphopenic mice, led to propose a major regulatory role of induced Tregs (iTregs) in microbiota-driven colonic inflammation (reviewed in[200]). It is, however, notable that selective depletion of mouse iTregs through inactivation of the Foxp3 intronic enhancer CNS1 did not induce colonic inflammation, while resulting in severe spontaneous TH2-mediated inflammation in the upper part of the gastrointestinal tract and in the lungs together with increased serum IgE and IgA.[201] Enhanced antibody response to chow antigens in $CNS1^{-/-}$ mice further suggested that iTregs protect against food allergy.[201] This conclusion is supported by the work of Hadis et al.[202] showing that oral tolerance to ovalbumin cannot establish upon $Foxp3^+$ Tregs depletion.

Clinical observations in patients with IPEX syndrome support the view that Tregs are essential to restrain autoimmunity and TH2 responses but are less crucial for tolerance to microbial antigens. Over 150 patients have been reported and two recent articles have summarized findings in 96[203] and 30[204] patients, respectively. In the majority of cases, symptoms start during the neonatal period with severe chronic diarrhea and extensive eczema-like lesions. Autoimmune disorders are frequent, most often type I diabetes but also hemolytic anemia, thrombocytopenia, thyroiditis and less commonly hepatitis, nephropathy, arthritis and/or lymphadenopathy. In some patients, disease develops later in life and sometimes with relatively mild symptoms. Mutations can affect all domains of the protein without any strong evidence for genotype–phenotype correlation. FOXP3 expression and numbers of $FOXP3^+$ $CD4^+CD25^+$ T cells are variable between patients but their suppressive function is impaired when they are present. Most patients require heavy immunosuppression and must be considered for HSCT. Yet rare asymptomatic or spontaneously resolutive cases have been recently reported.[203,204] Strikingly, histological lesions predominate in the small intestine with total or subtotal villous atrophy and strong "lamina propria" infiltration by lymphocytes and often eosinophils. Bloody diarrhea and macroscopic colitis are less frequent and rarely seen alone. In the most severely affected patients, extensive crypt and glandular destruction can, however, extend to the colon and mimic graft-versus-host disease. Oesophagitis and gastritis of variable severity are also possible.[205] Overall histological findings do not plead for microbiota-induced inflammation but are rather consistent with autoimmune and/or food-driven reactions (Fig. 6). Attesting intestinal self-reactivity, IPEX enteropathy is almost invariably associated with autoantibodies against IEC and notably against harmonin, a 75kD antigen expressed in the brush border of the small intestine.[203,204] As the latter antibodies can disappear under immunosuppression while severe lesions persist, their role in tissue damage is likely limited and lesions rather result from uncontrolled activation of self-reactive T cells. Of note, not only excessive TH1 but also TH2 responses can be demonstrated in intestinal biopsies[206] and, in keeping with observation in $CNS1^{-/-}$ mice, most patients display hyper-IgE[204] and over 30% develop severe allergy, notably food allergies attested by IgE antibodies against food allergens.[204,206] $FOXP3^+$ Tregs use multiple immunosuppressive mechanisms (reviewed in[200,207]). Their

production of IL-10 and TGF-β1 and their expression of CTLA-4 is thought to be important in mice to prevent colonic inflammation.[200,207] As discussed below, the role of CTLA-4 is important in humans.

Other T-cell-intrinsic gene defects impairing intestinal immunoregulation: Several gene defects impairing Tregs function and or enhancing effector T-cell functions can manifest by an IPEX-like disease with autoimmune enteropathy.

Loss-of-function mutations in CD25, CD122 and STAT5: IL2RA (CD25) deficiency, that leads in mice to impaired thymic development and peripheral homeostasis of Tregs,[208] has been described in a few patients with severe autoimmune enteropathy, massive infiltration of lymphoid and nonlymphoid organs by cytotoxic $CD8^+$ T cells but also severe viral infections (Fig. 6). Unexpectedly, the number of $CD4^+$ $CD127^{low}$ CTLA-$4^+$ $FOXP3^+$ T cells was close to normal. Since cytokines were increased in serum and circulating T cells showed increased proliferation.[209] It was suggested that, due to the lack of $CD25^+$ Tregs, more IL-2 was available and could drive non-cognate activation and expansion of tissue damage-causing $CD8^+$ T cells, while the lack of CD25 might impair priming of antiviral T-cell responses.[209] The paradoxical persistence of FOXP3 Tregs was explained by residual signaling via the CD122/CD132 complex in response to IL-2 and IL-15. Accordingly, a profound defect in Tregs was observed in patients with biallelic LOF mutations in *IL2RB* (CD122)[210] or in *STAT5B*,[211] a key transcriptional factor downstream the two cytokines. In keeping with the lack of Tregs, CD122-deficient patients developed dermatitis and autoimmune symptoms including enteropathy, cytopenia, thyroiditis and some had food allergy,[210] while celiac-like disease, autoimmune thyroiditis, inflammatory skin and lung lesions were reported in STAT5b-deficient patients[211] (Fig. 6). Importantly, CD122- and STAT5b-deficient patients, alike CD25-deficient patients, showed increased susceptibility to herpes virus and notably cytomegalovirus, underscoring the multifaceted role of IL-2 in immunity.[210,211] Due to impaired response to growth hormone, STAT5b-deficient patients also showed short stature.[211]

Loss-of-function mutations in CTLA-4 and LRBA: Treg dysfunction, IPEX-like disease and autoimmune enteropathy are also hallmarks of monoallelic LOF mutations (haploinsufficiency) in CTLA-4[212,213] and of biallelic recessive mutations in LRBA,[214–216] two proteins that act in concert (Fig. 6). CTLA-4, a well-known check-point inhibitor, is expressed by FOXP3 Tregs and activated T cells and inhibits immune responses by competing with CD28 for binding to CD80 and CD86 on antigen-presenting cells or by removing these two molecules by transendocytosis, thus allowing their lysosomal degradation. LRBA regulates the intracellular stock of CTLA-4. Indeed, CTLA-4 undergoes constant recycling to the cell surface and its interaction with LRBA in endosomes prevents its translocation and rapid degradation in lysosomes.[214] Strikingly, disease penetrance is variable and onset can be delayed in the second decade of life or even later.[213–217] While the numbers of Tregs is variable in CTLA-4- and LRBA-deficient patients, their expression of CTLA-4 and their suppressive functions are constantly impaired.[212,217] Treg impairment has therefore been proposed as the major cause of the severe autoimmune symptoms.[217] Yet, patients display additional symptoms absent in FOXP3 deficiency: they can notably develop severe lung interstitial pneumonia, hepatosplenomegaly and lymphadenopathy, granulomatous and lymphoid infiltration of the brain or kidney[213,215–217] as well as severe lymphocytic colitis, reminiscent of that observed in cancer patients treated by CTLA-4 antagonists.[218] It is tempting to suggest that the massive infiltration of both lymphoid and nonlymphoid organs reflects the unrestrained activation of conventional T cells lacking an intrinsic CTLA-4-/LRBA-dependent brake. Despite in vitro evidence that human conventional T cells lacking CTL-4 are hyperproliferative,[212,214] this hypothesis remains, however, disputed.[217] In addition, CTLA-4 and LRBA deficiencies are frequently associated with CVID and therefore with the infectious complications that can be associated with this condition (see above) (Fig. 6). Importantly, patients with CTLA-4 haploinsufficiency or LRBA LOF mutations can be dramatically improved by administration of CTLA-4-Ig (abatacept), a fusion protein composed of the extracellular domain of CTLA-4 and the Fc portion of IgG.[213–217] Through binding to CD80 and CD86 on antigen-presenting cells, CTLA-4-Ig blocks their interaction with CD28 on T cells, thereby limiting T-cell activation.[214] The use of hydroxychloroquine in order to limit lysosomal degradation of CTLA-4 and to enhance its surface expression has also been suggested.[214]

Mutations enhancing the JAK-STAT pathway: While the intrinsic T-cell defects discussed above impair intestinal immunoregulation exclusively or mainly via their impact on Tregs, other gene defects enhance effector T-cell activation. This is likely one mechanism by which "GOF mutations in *STAT3*" encoding Signal transducer and activator of transcription 3 can cause a broad and diverse spectrum of autoimmune diseases (e.g., diabetes, celiac-like enteropathy, cytopenia, thyroiditis, hepatitis, arthritis, scleroderma) as well as lymphoproliferation, interstitial pneumonitis, and dermatitis.[219–221] As in IPEX syndrome, intestinal T-cell infiltration is frequent and predominates in the small intestine although lymphocytic colitis can also develop[221,222] (Fig. 6). Patients also display increased susceptibility to viral, fungal, and bacterial infections. Respiratory bacterial infections can be further favored by the frequent development of hypogammaglobulinemia and CVID.[219–221] Age at onset, penetrance and immune symptoms are strikingly variable between patients even within the same family ([221] and personal observation), probably reflecting the need of a trigger to initiate inflammation. Indeed, STAT3 is best known for its transcriptional activity downstream a broad spectrum of cytokine receptors. Depending on the cytokine, receptor binding induces the recruitment and activation of one of the four Janus-Kinases JAK1–JAK3 and TYK2, which, in turn, phosphorylate STAT3, allowing its dimerization and nuclear translocation. In the nucleus, STAT3 binds to the canonical STAT sequence CCT(N)3GAA to activate or repress gene transcription and modulate activation, differentiation, and proliferation in many distinct immune cell subsets.[221,223] In contrast to many somatic *STAT3* GOF mutations observed in cancers, germinal *STAT3* GOF mutations do not result generally in constitutive STAT3 phosphorylation, but they enhance STAT3-dependent transcription in response to cytokines. The dependence on cytokine signals together with the very broad range of cytokines that can activate STAT3, including IL-6 and oncostatin M, γc cytokines, interferons, IL-10 and related cytokines, IL-12 and IL-23, likely contribute to the diversity of immune symptoms and to their variable onset. The role of individual effector T-cell subsets in tissue damage is not fully delineated. Excessive activation of TH17 cells may be involved in arthritis.[220] In contrast strong CD8+ T-cell infiltration and enhanced production of granzyme B and IFNγ were observed in the intestine.[222] Competition of STAT3 with other STATs for their common DNA binding site or excessive induction of common negative regulators such as SOCS3 may reduce STAT5 and STAT1 activation, thereby impairing Tregs function (reported in some studies) and resistance to viral and mycobacterial infection, respectively. Additional contribution of other transcriptional or non-transcriptional functions more recently ascribed to STAT3 is not excluded (reviewed in[221,223]). Yet, improvement or remission of autoimmune symptoms and notably of the enteropathy after treatment with ruxolitinib, a JAK1/2 inhibitor, strongly suggests that excessive activation the JAK/STAT pathway is instrumental.[222,224] In keeping with the role of somatic *STAT3*

GOF mutations in the emergence of large granular lymphocyte leukemia and lymphomas, patients are at risk to develop such malignancies.[220,225] The mechanism(s) of hypogammaglobulinemia observed in a large fraction of patients remain(s) more poorly understood inasmuch as STAT3 promotes the differentiation of follicular helper T cells and is downstream several cytokines which stimulate proliferation or differentiation of B cells (IL-6, IL-10, and IL-21).[226] Increased numbers of activated B cells but reduced numbers of switched memory B cells have been reported.[225] Substitution with immunoglobulins is necessary to reduce the risk of respiratory infections (Fig. 6).

Besides *STAT3* GOF mutations, "*STAT1* GOF mutations" have been identified in over 300 patients with autoimmunity, severe viral, bacterial and invasive fungal infections, aneurysms, and cancers. Intestinal inflammation has been reported in 4% of the patients and can manifest as IPEX-like disease, severe Crohn's like disease or even ulcerative colitis (Fig. 6). Development of Tregs does not seem to be affected and enhanced autoimmunity and inflammation are therefore ascribed to excessive interferon signaling.[227] Downregulation of the STAT3 pathway as the consequence of STAT1 hyperactivation severely impairs TH17 immunity, explaining the susceptibility to bacterial and fungal infections, including esophageal candidiasis. Autoimmune symptoms are often associated with autoantibodies, while memory B cells, IgG2, and IgG4 are frequently decreased.[227]

The need of tight regulation of the JAK-STAT pathway to preserve intestinal homeostasis is further illustrated by two recent reports. Thus, a "de novo mosaic mutation in the pseudokinase domain of JAK1" was identified in one patient, who developed very early in life a complex syndrome combining severe diffuse gastrointestinal inflammation, skin lesions and nephrotic syndrome. The mutation increased JAK1 activity but also transactivated partner kinases JAK2, TYK2, and JAK3, resulting in the activation of STAT1, 3, and 5 by multiple cytokines in the cells carrying the mutation.[228] Treatment with the JAK1/3 inhibitor tofacitinib allowed clinical resolution. Finally, we have recently reported the onset of severe autoimmune enteropathy in a 3-month girl carrying a LOF mutation "in the tyrosine-protein phosphatase non-receptor type 2 PTPN2" and shown that, in humans this enzyme selectively controls the activation of the JAK-STAT pathway and notably STAT3 activation[141] (Fig. 6).

## CONCLUSIONS AND PERSPECTIVES

Thanks to next generation sequencing, identification of Mendelian disorders that affect the epithelial and or the hematopoietic cellular components of the human intestinal barrier is rapidly progressing, providing an increasingly detailed roadmap of genes that are indispensable to maintain intestinal homeostasis in humans. While animal models, and notably mouse models, are essential to gain mechanistic insight into the role(s) of individual genes, gene functions can differ between species and genetic dissection of monogenic diseases is necessary to delineate redundancy and to hierarchize gene contribution to the proper function of the human intestinal barrier. One first interesting observation thus relates to the compartmentalization of the immune mechanisms that maintain homeostasis in the upper and lower part of the intestine, which was not fully anticipated from mouse studies. As highlighted in this review and observed through the genetic analysis of cohort of over 500 children with very early onset intestinal inflammation ([17] and unpublished results), inflammation in the proximal small intestine is mainly observed in defects impairing regulation of adaptive immune responses, while ileocolonic inflammation is mainly associated with defects impairing phagocytic functions or regulation of innate immune cells. These location patterns likely reflect differences in the mechanisms evolved by the host to cope with immune stimuli present in the upper part of the intestine (mainly food antigens and autoantigens) and in its lower part dominated by microbiota. A second observation concerns the variability in presentation and penetrance of many immune defects, which raise puzzling questions on the underlying mechanism(s). An important contribution of environmental trigger(s) is plausible. Along this line, a continuum between Mendelian and common complex forms of IBD is likely. Thus, it is notable that we could identify a gene defect in 60% of very young children with small intestinal autoimmune-like inflammation, but only in 15% of those with exclusively colonic inflammation ([17] and unpublished results). In addition, there is a substantial overlap between gene pathways identified by the study of Mendelian forms of IBD and those highlighted by GWAS in common forms of IBD (bioRxiv 768028; https://doi.org/10.1101/768028). Further studies will be useful to delineate precisely the overlap between rare and common forms of IBD and define how it can be leveraged to improve understanding and treatment of the latter diseases.

Beyond providing insight into the mechanisms of the human intestinal barrier, identification of gene defects causing intestinal inflammation is a key to guide therapy. Except for intestinal transplantation, which remains with very low rate of success,[229] definitive therapeutic options are still lacking for diseases that severely affect epithelial differentiation. As mentioned above, pharmacological approaches to restore a functional epithelium are currently under study.[62,64] Important efforts are also invested by several groups in gene editing and tissue engineering.[230,231] In contrast, there are now many therapeutic options for diseases that impair exclusively or predominantly the hematopoietic compartment. Besides HSCT, which can definitively cure the most severe diseases, gene therapy is under development for several immune defects. Moreover, targeted treatments are now available for diseases that impair CTLA-4-dependent regulation or lead to excessive activation of the inflammasome or of the JAK-STAT pathway (see above). Several of these drugs are currently under consideration to treat common forms of IBD. Yet, given disease heterogeneity, therapeutic benefit is expected to vary between patients and one major challenge is to identify markers that may guide the choice of the best personalized therapy. One attractive hypothesis is that signatures characteristic of individual pathways dysregulated in monogenic disorders might be used to stratify patients with common forms of IBD. Designing such studies might help to bridge the gap between rare and common forms of IBD and should provide precious tools for precision medicine in IBD.

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## AUTHOR CONTRIBUTIONS

F.C.-H., M.P., and N.C.-B. wrote the article. M.P. and N.C.-B. designed the figures. M.P., G.M., and F.R. reviewed the paper.

## ADDITIONAL INFORMATION

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