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Translating Immunology into Therapeutic Concepts for Inflammatory Bowel Disease

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Abstract

Inflammatory bowel disease (IBD) defines a spectrum of complex disorders. Understanding how environmental risk factors, alterations of the intestinal microbiota, and polygenetic and epigenetic susceptibility impact on immune pathways is key for developing targeted therapies. Mechanistic understanding of polygenic IBD is complemented by Mendelian disorders that present with IBD, pharmacological interventions that cause colitis, autoimmunity, and multiple animal models. Collectively, this multifactorial pathogenesis supports a concept of immune checkpoints that control microbial-host interactions in the gut by modulating innate and adaptive immunity, as well as epithelial and mesenchymal cell responses. In addition to classical immunosuppressive strategies, we discuss how resetting the microbiota and restoring innate immune responses, in particular autophagy and epithelial barrier function, might be key for maintaining remission or preventing IBD. Targeting checkpoints in genetically stratified subgroups of patients with Mendelian disorder–associated IBD increasingly directs treatment strategies as part of personalized medicine.

INFLAMMATORY BOWEL DISEASE IS A CHRONIC DISORDER WITH MULTIFACTORIAL ORIGIN

Sufficient epithelial barrier function as well as innate and adaptive immune regulation is required for a lifelong balanced response to dietary antigens as well as bacteria, viruses, fungi, and parasites that colonize or infect the intestine. If those evolutionarily adapted mechanisms fail because of changes in lifestyle and environment, because of accumulation of common genetic susceptibility variants, or because of the occurrence of rare genetic defects with high functional impact, chronic intestinal inflammation can arise. Inflammatory bowel disease (IBD) encompasses a group of complex disorders with three main phenotypes—Crohn's disease (CD), ulcerative colitis (UC), and IBD unclassified (IBDU). These disorders have a multifactorial etiology (1–3) and are a substantial health care problem with increasing incidence and prevalence worldwide (4). IBD is characterized by chronic relapsing disease activity of acute flares and intervals of remission (1, 2, 5). Sustained chronic intestinal inflammation causes tissue damage over time including fistulizing and stricturing disease in CD and life-threatening episodes of acute severe UC. Treatments for patients with IBD include anti-inflammatory, immunomodulatory, and immunosuppressive drugs as well as biologic therapies that target inflammatory cytokines such as tumor necrosis factor (TNF) or that impede immune cell homing, as recently reviewed by Neurath (6). Although many patients respond to frontline therapies, treatments need to be escalated in a substantial proportion of patients, and primary or secondary nonresponse is observed. Long-term effects caused by uncontrolled inflammation include cancer, and there are side effects of current treatments such as myelotoxicity, sepsis, or reactivation of infections. There is a need to develop targeted medications that are based on pathogenic mechanisms, are disease subtype- and organ-specific, and are associated with reduced side effects. To achieve this goal, it is important to understand the immunobiology of IBD, to differentiate subgroups of patients on the basis of biomarkers, and to focus novel treatments based on a molecular process–driven taxonomy.

In this review, we discuss cellular components of the immune system that maintain barrier function. We also discuss dysregulated molecular signaling networks that disrupt immune homeostasis as a consequence of susceptibility mechanisms that underlie classical polygenic IBD, Mendelian disorders in humans, adverse events, or targeted manipulation in animal models (**Figure 1**). Developing novel medications and interventions that target key checkpoints offers the opportunity to treat and prevent IBD. By focusing on IBD pathogenesis, we do not discuss how genetics affects drug responses and adverse events in IBD, i.e., the emerging field of pharmacogenetics.

GENETICS OF INFLAMMATORY BOWEL DISEASE

Drug Targets Informed by Common Genetic Variation

Genome-wide association studies (GWASs) have identified more than 230 loci linked to human IBD (7–9). Of these variants, only a minority are protein coding, and the majority are intronic and intergenic loci (3, 10). Candidate genes within these loci suggest a role for the epithelial barrier, innate immune responses, and adaptive immune dysregulation (1, 2). Association studies have generated information on host-microbe interactions via the NOD2 pathway, identified autophagy as a pathogenic mechanism in CD, and supported the role of IL-23-driven Th17 cell responses (3, 7–10). They have also highlighted similarities and differences in IBD susceptibility among ethnicities (7, 8, 11) and in comparison with other inflammatory disorders such as ankylosing spondylitis, psoriasis, and primary sclerosing cholangitis (12–15). The IBD risk variant burden of all CD- or UC-associated loci allows statistical discrimination among CD, IBDU, and UC phenotypes (16), placing IBDU as a subgroup between colonic CD and UC in terms of variant burden.

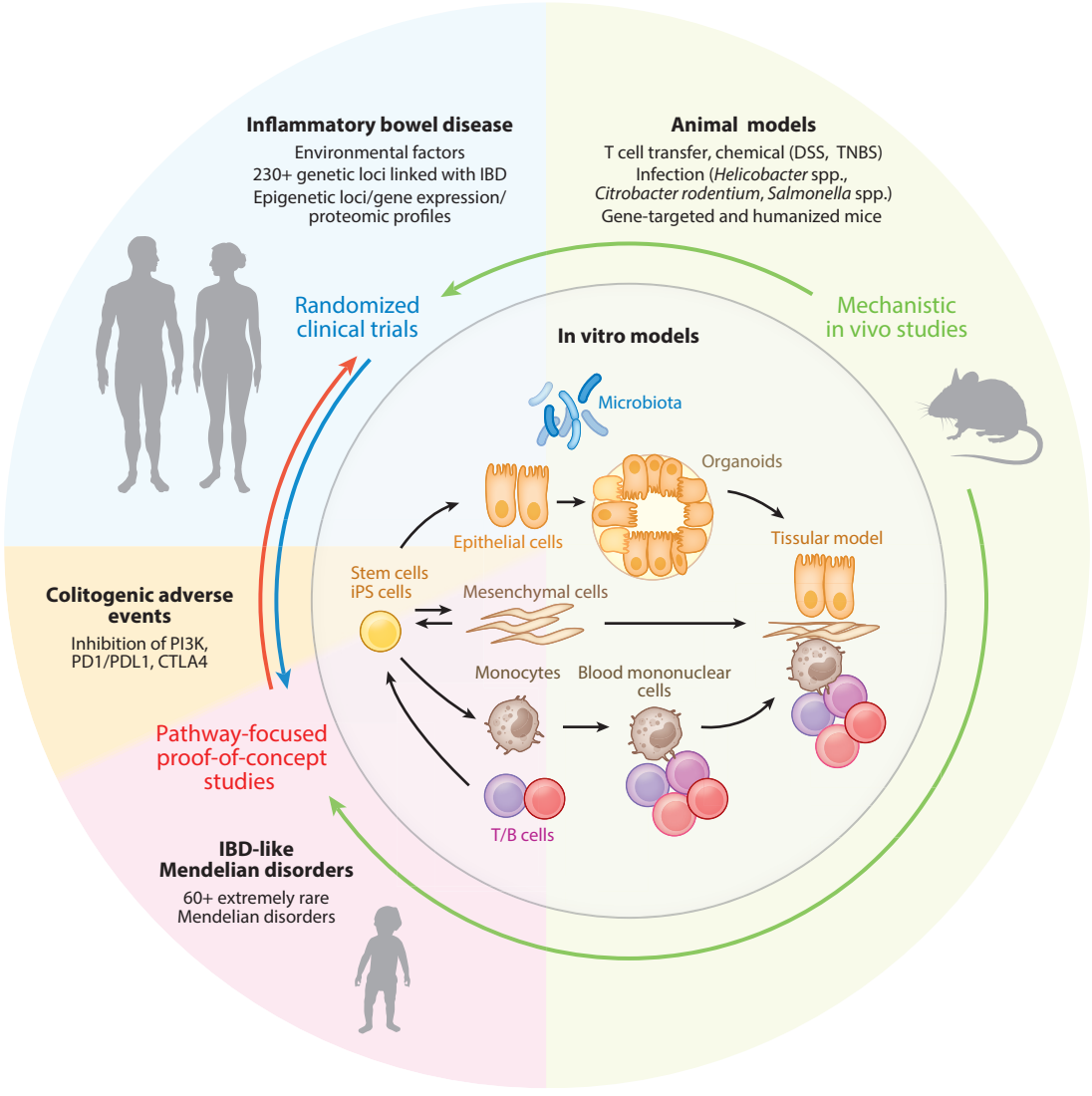


Figure 1

Understanding immune mechanisms that drive intestinal inflammation in humans and mice. Epidemiological findings, genetic associations, clinical observations, and therapeutic interventions in IBD patients inform our understanding of immunological mechanisms in classical IBD. This is further complemented by understanding Mendelian disease–associated IBD and adverse effects of therapeutic interventions that can induce intestinal inflammation. Animal models reflect aspects of IBD phenotypes. Increasingly complex in vitro models help in understanding cellular or tissular aspects of the epithelial, mesenchymal, and immune phenotypes. Data derived from diverse model systems provide information on mechanisms and preclinical drug targets. Abbreviations: DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; iPS, induced pluripotent stem cell; TNBS, 2,4,6-trinitrobenzenesulfonic acid.

Most IBD susceptibility loci contain multiple genes (7–9, 17), and further fine mapping is required. Eighteen of the IBD-associated loci are mapped with high confidence to a single variant (17). These include protein coding variants in *NOD2*, *IL23R*, *CARD9*, *SMAD3*, and *IFIH1*; intronic variants in the locus of *IL2RA*, *LRRK2*, *NOD2*, *HNF4A*, and *RTEL1/TNFRSF6B*; and intergenic variants in the locus of *PRDM1*, *IKZF1*, *JAK2*, *NKX2-3*, and *HNF4A* (17). However, it

is not clear to what extent the majority of GWAS loci with low-association signals can inform functional signaling pathways because minute associations have been revealed by an ever-increasing number of patients, resulting in a genome-spanning omnigenic model (18). Whereas most studies have investigated disease susceptibility and not disease course, a recent study identified four variants associated with disease prognosis, suggesting that disease susceptibility and CD progression are determined by different genetic mechanisms (19).

Expression quantitative trait loci (eQTL) studies suggest that noncoding IBD loci are enriched within cell-specific enhancer regions that control the expression of genes in a cell-specific manner (20, 21). In addition to *cis*- and *trans*-acting eQTL where each variant affects a single or a small number of genes, genetic variants impacting the expression level of transcriptional regulators such as BACH2 can affect a large number of genes (superenhancer) (22). Multiple IBD eQTL have been described in peripheral blood, intestine, and immune cell subsets (23–25). Individual stimulation conditions determine eQTL effects, and opposing transcriptional effects of one and the same variant across different cell types have been described (20). In addition to genomic variation, epigenetic modification affects transcription. Epigenetic marks are specific to cell types such as epithelial cells, monocytes, or T cells, and they enrich within regulatory regions identified by IBD GWASs (26–28). eQTL and methylation quantitative trait studies help to explain the large noncoding variation observed, and they link the genetic association signal with directionality of mRNA expression. In light of these tissue-specific regulatory regions and epigenetic modification, it makes sense that transcriptional risk scores based on eQTL variants associated with IBD and linked to RNA-sequencing gene-expression data outperformed genetic risk scores in differentiating CD from controls. These transcriptional risk scores also identified patients with subsequent complicated disease course (29).

Several genetic associations include candidate genes in therapeutically relevant immune signaling pathways. The important therapeutic role of the IL-23 pathway was initially informed by the protective p.V362I variant in *IL23R* (discussed in detail below) (30). Similarly, inhibition of immune cell homing by blocking the $\alpha 4\beta 7$-integrin as a successful therapeutic concept in patients with UC and CD (31, 32) is supported by not only mechanistic studies (33) but also genetic association signals (9). The therapeutic value of blocking specific immune cell homing pathways is further reiterated since blocking mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1), an endothelial ligand that attracts mucosal homing integrin $\alpha 4\beta 7$ cells, has shown efficacy in UC (34) although not in CD (35). Those findings are in line with the emerging concept in biomedicine that medications supported by genetic signals are more successful in the clinic. There is now a spectrum of promising genetically informed drug target candidates that might be of therapeutic relevance in IBD such as the Th17 cell–defining transcription factor *RORC* (9) or a recently identified loss-of-function variant in the *RNF186* gene that confers protection against UC (36).

The combination of protein coding variants, epigenetic association marks, and eQTL allows for construction of models of cellular and molecular networks (37) associated with IBD pathogenesis. These networks may predict therapeutic interventions that correct perturbed signaling pathways instead of targeting the modest effects of individual variants.

Mendelian Disorder–Associated Inflammatory Bowel Disease

In addition to the classical forms of IBD, Mendelian disorders can present with IBD-like intestinal inflammation (MD-IBD) (38, 39). Patients with MD-IBD often present with extreme phenotypes such as infantile-onset IBD, infections due to immunodeficiencies, or a range of other extraintestinal manifestations. Because these disorders are typically caused by protein coding defects,

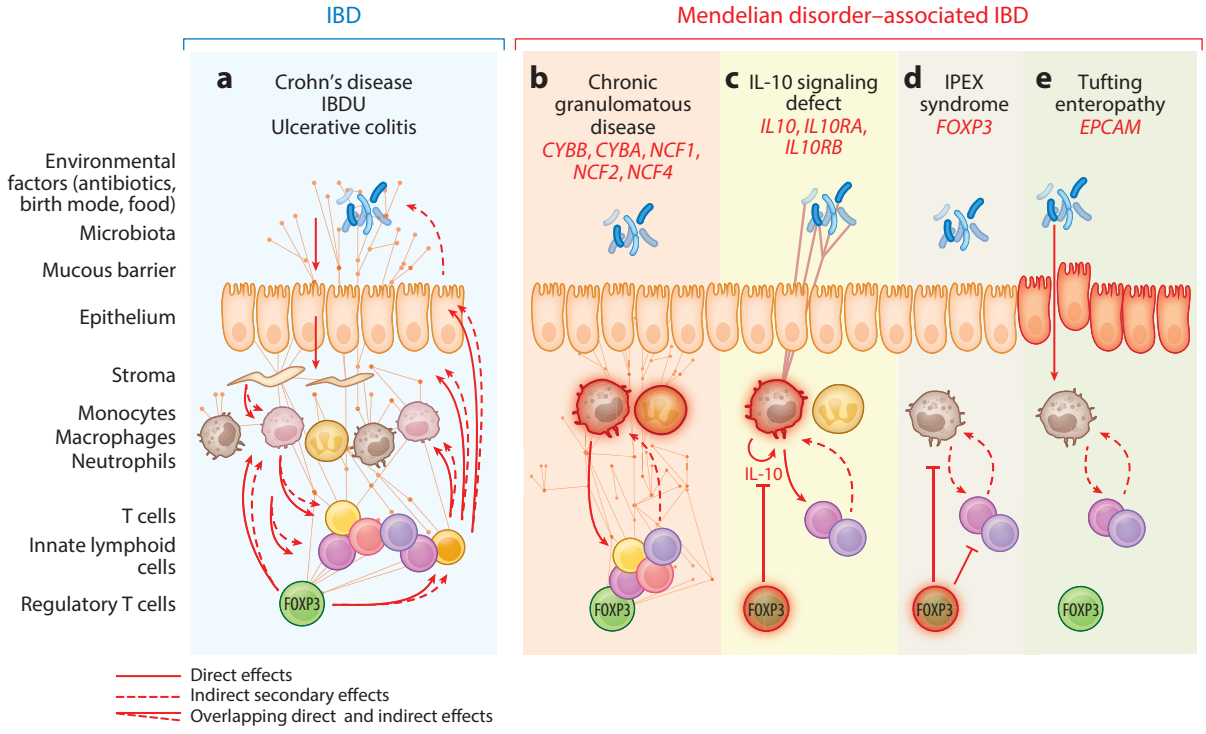
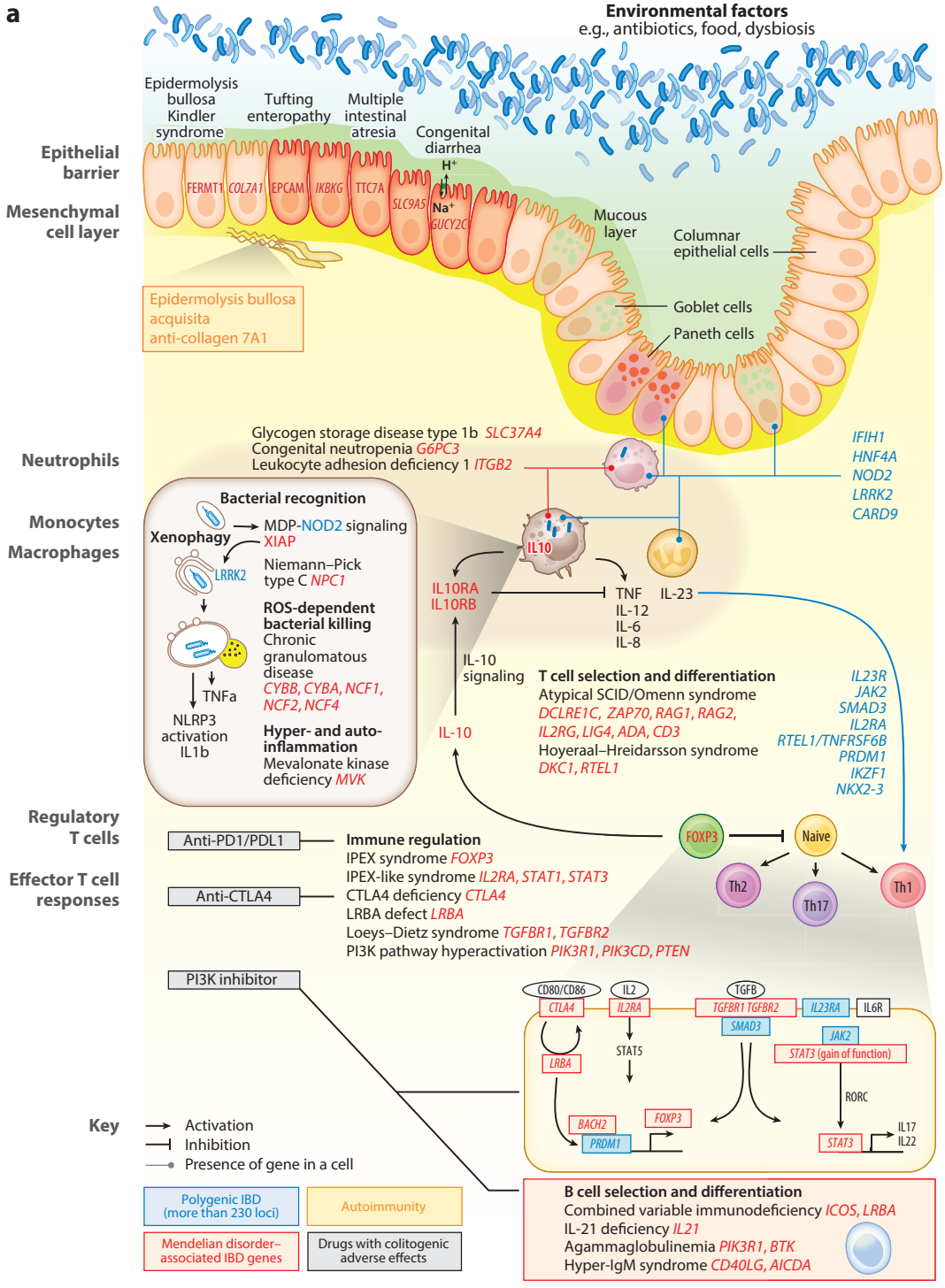


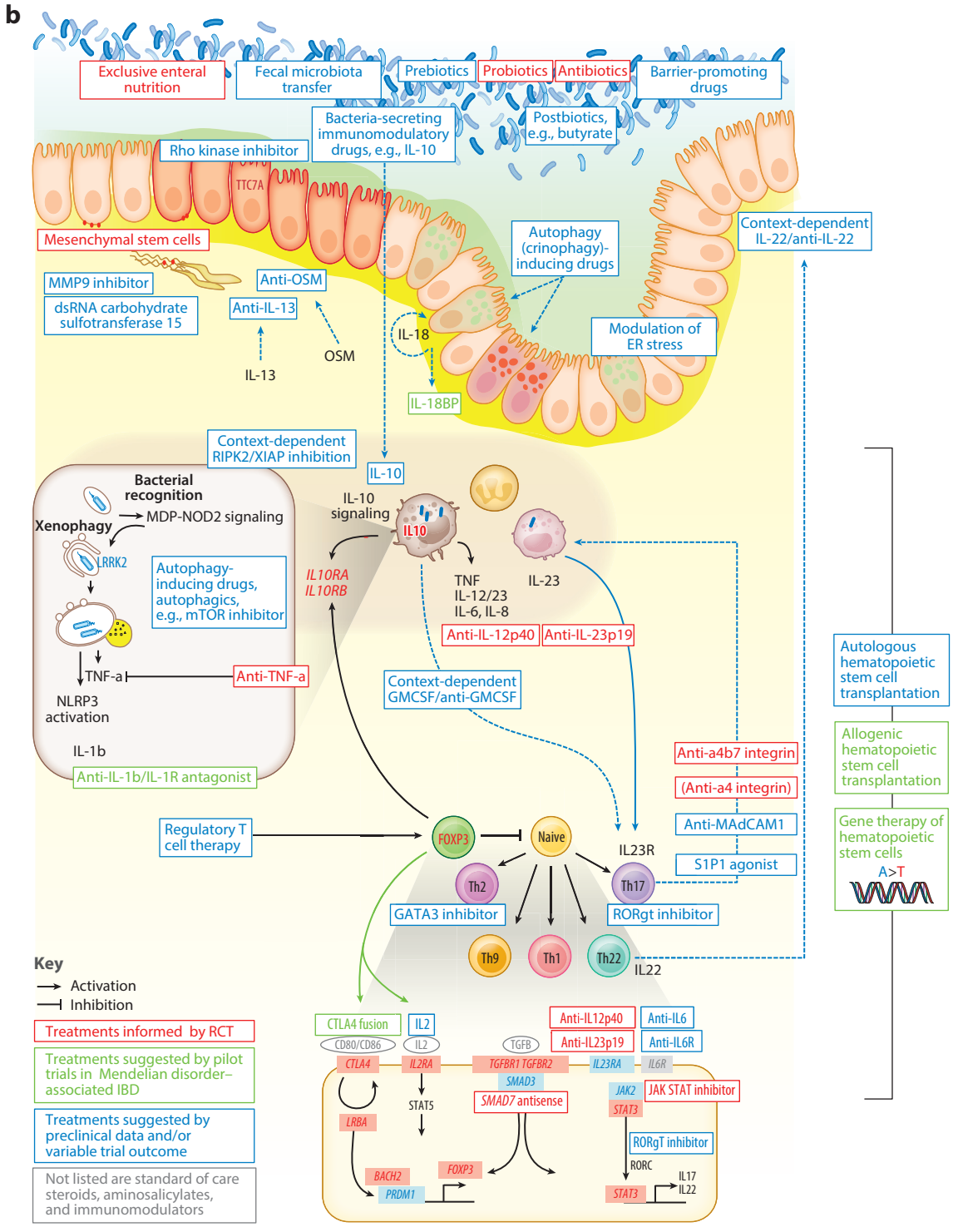
Figure 2

Diverse mechanisms drive intestinal inflammation in polygenic IBD as well as MD-IBD with similar histological outcomes. (*a*) In complex polygenic IBD, multiple genetic factors with low or moderate impact affect multiple layers of the mucosal immune response including epithelial cells, mesenchymal cells, phagocytes, regulatory cells, T cells, and B cells. An interaction network links environmental factors with dysbiosis of the microbiota, epithelial barrier function, and immune networks, resulting in multiple primary (*solid lines*) and/or secondary (*dotted lines*) responses. (*b*) Mutations in the NADPH oxidase complex disrupt bacterial handling in phagocytes and result in chronic granulomatous disease. The genetic background of IBD susceptibility genes contributes to the IBD phenotype. Adaptive immune activation is a secondary consequence of the disturbed innate immune defect. (*c*) Loss-of-function defects in IL-10 signaling cause infantile-onset IBD with complete penetrance. In these patients there is either loss of macrophage responsiveness to IL-10 due to defects in the IL-10 receptor or deficiency of IL-10 production by monocytes/macrophages and regulatory T cells. The contribution of common IBD susceptibility factors in IL-10 signaling defects is unlikely, but the presence of intestinal microbiota may be required for development of IBD. (*d*) Loss of regulatory FOXP3$^+$ T cell activity causes dysregulated innate and adaptive immune responses in patients with IPEX syndrome. (*e*) Defects in the epithelial gene *EPCAM* causes epithelial cell damage and tufting enteropathy. Some patients with an otherwise functional immune system develop intestinal inflammation that is most likely caused by increased translocation of the microbiota and subsequent innate and adaptive immune activation. Abbreviations: IBD, inflammatory bowel disease; IBDU, IBD unclassified; IPEX, immunodysregulation polyendocrinopathy enteropathy X-linked; MD-IBD, Mendelian disorders with IBD-like intestinal inflammation.

information about them can further our understanding of functional mechanisms that affect the layers of the intestinal immune system.

Among the 60 MD-IBD genes, IL-10 signaling defects caused by loss-of-function mutations in *IL10*, *IL10RA*, and *IL10RB* genes are the strongest factors that lead to infantile IBD (40, 41). Other defects cause dysfunctional regulatory T cell activity, disrupt T and B cell selection and activation, reduce clearance of bacteria by neutrophil granulocytes or macrophages, cause autoinflammatory responses, or affect epithelial barrier function (38, 39). This supports a model wherein multiple Mendelian-type defects with high functional impact on different cellular components can independently cause—either directly or indirectly—innate and adaptive immune activation (**Figure 2**).


a
Environmental factors
e.g., antibiotics, food, dysbiosis
Epithelial barrier
Mesenchymal cell layer
Neutrophils
Monocytes
Macrophages
Regulatory T cells
Effector T cell responses
Epidermolysis bullosa Kindler syndrome
Tufting enteropathy
Multiple intestinal atresia
Congenital diarrhea
FERMT1
COL7A1
EPCAM
IKBKG
TTC7A
SLC9A5
GUCY2C
H+
Na+
Mucous layer
Columnar epithelial cells
Goblet cells
Paneth cells
Epidermolysis bullosa acquisita anti-collagen 7A1
Glycogen storage disease type 1b SLC37A4
Congenital neutropenia G6PC3
Leukocyte adhesion deficiency 1 ITGB2
IFIH1
HNF4A
NOD2
LRRK2
CARD9
Bacterial recognition
Xenophagy
MDP-NOD2 signaling
XIAP
LRRK2
Niemann–Pick type C NPC1
ROS-dependent bacterial killing
Chronic granulomatous disease
CYBB, CYBA, NCF1, NCF2, NCF4
TNFa
NLRP3 activation IL1b
Hyper- and auto-inflammation
Mevalonate kinase deficiency MVK
IL10
IL10RA
IL10RB
TNF
IL-12
IL-6
IL-8
IL-23
IL-10 signaling
IL-10
T cell selection and differentiation
Atypical SCID/Omenn syndrome
DCLRE1C, ZAP70, RAG1, RAG2, IL2RG, LIG4, ADA, CD3
Hoyeraal–Hreidarsson syndrome
DKC1, RTEL1
IL23R
JAK2
SMAD3
IL2RA
RTEL1/TNFRSF6B
PRDM1
IKZF1
NKX2-3
FOXP3
Naive
Th2
Th17
Th1
Anti-PD1/PDL1
Anti-CTLA4
Immune regulation
IPEX syndrome FOXP3
IPEX-like syndrome IL2RA, STAT1, STAT3
CTLA4 deficiency CTLA4
LRBA defect LRBA
Loeys–Dietz syndrome TGFBR1, TGFBR2
PI3K pathway hyperactivation PIK3R1, PIK3CD, PTEN
PI3K inhibitor
CD80/CD86
CTLA4
IL2
IL2RA
TGFB
TGFBR1 TGFBR2
IL23RA
IL6R
SMAD3
JAK2
STAT3 (gain of function)
LRBA
STAT5
RORC
BACH2
PRDM1
FOXP3
STAT3
IL17
IL22
B cell selection and differentiation
Combined variable immunodeficiency ICOS, LRBA
IL-21 deficiency IL21
Agammaglobulinemia PIK3R1, BTK
Hyper-IgM syndrome CD40LG, AICDA
Key
Activation
Inhibition
Presence of gene in a cell
Polygenic IBD (more than 230 loci)
Autoimmunity
Mendelian disorder–associated IBD genes
Drugs with colitogenic adverse effects



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding pages*)

(*a*) Pathogenic mechanisms and targeted therapies. Diverse functional mechanisms drive intestinal inflammation in polygenic IBD, MD-IBD, autoimmunity, and therapy-induced IBD (adverse events). All those diverse mechanisms result in a spectrum of similar histological outcomes reflecting Crohn's disease, ulcerative colitis, and IBDU. The intestinal mucosal barrier is maintained by several cell types including epithelial cells, mesenchymal cells, neutrophils, monocytes/macrophages, and lymphocyte subsets. Major cell subsets with a likely pathogenic role as suggested by genetic information, adverse events, and autoimmunity are shown. In polygenic IBD, 230 established genetic loci are associated with IBD; shown in blue are those that are resolved to single a variant (17). Genetic defects in MD-IBD are depicted in the category in which the main functional defect likely operates (*red*). Defects in a number of genes affect multiple different cells within the mucosal defense system (e.g., *NOD2* in epithelial cells and several innate immune cells). (*b*) Therapeutic interventions target dysregulated pathogenic networks or aim to compensate factors that are deficient. Those treatments are partially informed by (multiple) RCTs in IBD (the desired standard; *red*). Treatments suggested by pilot interventions in Mendelian disorders may have strong effect size and are highly informative but in most instances require follow-up studies (*green*). The potential benefit of additional targets suggested by preclinical models and/or interventions with less uniform trial outcomes in patient groups need to be further evaluated (*blue*). The different interventions are shown in relation to the likely pathomechanism that they target. Hematopoietic stem cell transplantation and gene therapy of hematopoietic stem cells replace a large array of innate and adaptive immune cells (*parentheses*). Abbreviations: IBD, inflammatory bowel disease; IBDU, IBD unclassified; MD-IBD, Mendelian disorders with IBD-like intestinal inflammation; OSM, oncostatin M; RCT, randomized controlled trials; ROS, reactive oxygen species.

Whereas IL-10 signaling defects confer complete penetrance, most MD-IBD genes are likely not strictly monogenic, but instead act on a background of shared genetic IBD susceptibility and unresolved environmental factors. For example, approximately 30% of patients with chronic granulomatous disease develop IBD. Mutations in the NADPH oxidase genes (*CYBB*, *CYBA*, *NCF1*, *NCF2*, or *NCF4*) constitute the pathogenic driver for chronic granulomatous disease immunodeficiency, whereas variants in additional IBD-risk loci modify susceptibility to intestinal inflammation (42).

Clinical genomics, i.e., targeted panel sequencing as well as exome and genome sequencing, now provides a diagnostic standard of clinical care for identification of MD-IBD pathogenic variants (43). Identifying disease-causing defects not only explains the immunopathology, but also opens possibilities for pathway-specific therapies to correct the consequences of genetic defects, thereby enabling personalized medicine. A summary of immune pathways informed by Mendelian immune variants is provided in **Figure 3**.

Patients with Mendelian disorders are in exceptional need because many present with a complicated course. Owing to the rarity of these disorders, no formal assessments of treatments such as randomized controlled trials are available in most cases. Drugs are often used off-license without formal approval on the basis of retrospective uncontrolled case reports or series. These studies often represent an *n* of one case report and are prone to error, raise ethical considerations, and need validation in high-quality randomized controlled trials.

Despite these limitations, there are notable successes. Beyond the value to individual patients, proof-of-concept trials in MD-IBD provide an opportunity to generate and test hypotheses to identify interventions with strong effects in a small group of patients. For example, targeting IL-1 using the IL-1 receptor antagonist Anakinra or an anti-IL1b antibody has been effective in MD-IBD patients with primary inflammasome activation. This demonstrates that blocking the IL-1 signaling pathway in a primary autoinflammatory disorder such as Mevalonate kinase deficiency can resolve colitis (44). Application of Abatacept, a CTLA4 fusion protein, compensated for defective CTLA4 expression in patient cells with *CTLA4* variants in vitro (45, 46) and may be beneficial for patients with immune dysregulation (47). In patients with lipopolysaccharide-responsive and beige-like anchor protein deficiency, which causes defective CTLA4 endosomal vesicular trafficking within $FOXP3^+$ regulatory T cells and activated T cells, administration of the CTLA4 fusion protein restored immune homeostasis (48).

It is important to note that several medications that were postulated to be potentially helpful in MD-IBD subsets have failed in randomized controlled trials in IBD. These include the CTLA4 fusion protein Abatacept in CD and UC (49). However, functional stratification of patients with classical IBD may reveal subgroups of patients who could benefit from these treatments in the future.

ANIMAL MODELS OF INTESTINAL INFLAMMATION

Animal models are important for the mechanistic understanding of systemic and mucosal immune responses since they allow genetic manipulation, dissection of cellular compartments, and testing of therapeutic concepts. Translation of animal models into clinical practice depends on how well the model type reflects its associated human IBD disease type and state of chronicity. Due to the relative similarity between humans and mice and the availability of genetic tools, mice are the current standard for drug tests in IBD. By contrast, invertebrate (such as *Caenorhabditis elegans* or *Drosophila*) and small vertebrate (such as zebrafish) models have limitations beyond drug screening. In the last 25 years, hundreds of mouse models of intestinal inflammation have been studied, including infection models (such as *Salmonella* spp., *Citrobacter rodentium*, and *Helicobacter hepaticus*), chemically induced models (such as dextran sulfate sodium, 2,4,6-trinitrobenzenesulfonic acid, and oxazolone), and immune activation-induced (T cell transfer) models (50). In addition, more than 70 models use conditional mice and involve gene overexpression or deletion to target signaling or effector mechanisms either in all cells or specific cell types (51). In many cases, animals with genetic defects do not develop colitis spontaneously but reveal a functional role after infection or chemical challenge, supporting the concept of colitogenic gene-environment interactions.

Similar to Mendelian disorders in humans, mouse models confirm that extreme defects in different immune checkpoints and immune layers can cause intestinal inflammation. Many animal models with genetic manipulation mirror extreme phenotypes of patients with corresponding MD-IBD. A commonly used mouse model such as the T cell transfer model most closely reflects the situation found in atypical SCID (severe combined immunodeficiency) patients where a hypomorphic selection defect in T cells results in oligoclonal expansion and subsequent intestinal inflammation. Although reductionist, this T cell–driven pathology model depends on innate effector functions, and several aspects are relevant to human immunopathology (52). However, it is still a matter of debate how well many mouse models reflect classical human IBD. As such, ileal models with stricturing and fistulizing disease that reflect human CD features are underrepresented (53). Humanized mice constitute an interface between mouse and human immunology since they enable engrafting with human $CD34^+$ hematopoietic stem cells, allowing the study of pathogenic immune responses as shown for IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked) immune cells (54).

In addition to providing mechanistic insights, mouse models have been instrumental in selecting drug candidates. Before or parallel to transition into humans, animal models have informed therapies that have made it into the clinic, i.e., anti-TNF, anti-IL-23p40, anti-a4-integrin, anti-a4b7 integrin, and anti-IL-23p19, (50, 51). However, multiple drug candidates that prevented colitis in mouse models were ineffective in clinical trials, in part because most models have focused on prevention, not cure, of colitis and—most importantly—owing to the difficulty of relating models to appropriate patient subgroups, in particular differentiating between CD and UC. Mouse models are subject to significant variation both within and between models. Thus, there is currently no standard mouse model accepted by the US Food and Drug Administration or European Medicines Agency.

All combined, the data suggest that therapeutic efficacy in a mouse model of intestinal inflammation is an important but not sufficient surrogate for efficacy in humans. In addition, mouse

models to date do not allow good prediction of whether and in which human disease subset medications will be successful.

PHARMACOLOGICAL ADVERSE EVENTS THAT CAUSE INTESTINAL INFLAMMATION

Although much information has been gathered from successful targeted treatments in humans, treatment adverse events also provide important insights. Indeed, some of the most informative evidence comes from colitogenic adverse events. Checkpoint inhibitors have been developed to interfere with immune tolerance mechanisms to break tolerance to tumors. Treatments targeting PD1-PDL1, CTLA4, or PI3K signaling have successfully induced CD4 and CD8 T cell responses in a variety of malignancies (such as malignant melanoma) but have resulted in autoimmunity and intestinal inflammation (55, 56). The adverse event autoimmune profile observed by targeting CTLA4 or PI3K pharmacologically has strong similarity to the profile seen in MD-IBD and mouse models with distinct gene defects. For instance, the pathology caused by anti-CTLA4 (57) is reminiscent of patients with genetic defects in *CTLA4*, which causes an immune dysregulation syndrome with hypogammaglobulinemia, recurrent infections, and multiple organ autoimmunity including inflammatory enteropathy and CD-like lesions (45, 46, 58). This concept has considerable implications for anticipating intestinal inflammation as an adverse event since several proposed small-molecule inhibitors with anti-inflammatory activity target gene products associated with MD-IBD (such as *ADAM17*, MALT1, or XIAP) (59–61). However, predicting response and side effect profiles with expected certainty is a challenge because pharmacological inhibition is typically temporary and not complete.

PRECLINICAL IN VITRO MODELS OF INFLAMMATORY BOWEL DISEASE

The need to test adequate drug candidates requires robust and predictive preclinical models to test disease pathways in human cells. Advances in culturing primary cells, organoids, and complex tissular cultures together with advances in bacterial classification and manipulation have driven research toward novel directions. Further complementing such approaches are induced pluripotent stem cell technologies that allow generation of multiple cell types and opportunities arising from genetic manipulation of primary cells using CRISPR/CAS9.

Epithelial biology has been revolutionized by organoid technologies enabling growth of different subsets of human patient-derived small and large bowel epithelial cells including columnar epithelial cells, Paneth cells, and goblet cells. Organoid cell technologies replicate defects in intestinal epithelial function in vitro, as shown by the defective polarization of epithelial cells from patients with defects in TTC7A (62), a Mendelian disorder with increased susceptibility to intestinal inflammation. Culturing patient-derived epithelial cells facilitates understanding of mechanisms and screening of drug targets, as illustrated by reversal of the in vitro polarization phenotype seen in TTC7A-defective cells using a Rho kinase inhibitor (62). One problem with studying individual cell types is the lack of diverse cell-cell interactions since cytokine responses induce complex activation circuits beyond the initial signal. Development of complex tissular models such as the "gut on a chip" composed of epithelial cells, a connective tissue scaffold, and the immune system may potentially overcome those problems (63). Finally, advanced genomic technologies have allowed for the identification of new bacterial strains and species, and new techniques have facilitated culturing of previously challenging-to-cultivate bacteria, particularly anaerobic species (64).

CHECKPOINTS OF INTESTINAL INFLAMMATION AND THERAPEUTIC CONCEPTS

Proper intestinal functioning involves complex interactions between host and environmental factors. Central to this response is the functioning of immune cells and intestinal tissue resident cells including epithelial and mesenchymal cells. In the following, we focus on functional cellular and molecular checkpoints that confer susceptibility to intestinal inflammation in humans and animal models with a particular focus on those that present therapeutic opportunities.

Epithelial Barrier Function

Intestinal epithelial cells (IECs) and mesenchymal cells are key for intestinal barrier function and for the host response to infection and tissue damage. Iterative dialogue between the immune system and the IEC/mesenchymal cell unit ensures an effective homeostatic and inflammatory response including barrier maintenance and host defense. Although these interactions are host protective, accumulating genetic and biological evidence points to their subversion in IBD and highlights strategies that promote barrier function as therapeutic interventions.

The intestine is lined by a monolayer of columnar epithelial cells forming a barrier to microbes and noxious agents through the formation of tight junctions between cells (65, 66). As the first point of contact with the environment, IECs function not only as a stout barrier but also as an initiator of the innate immune response to pathogens and tissue damage. Specialized epithelial cells termed Paneth cells produce antimicrobial peptides within epithelial crypts, and goblet cells distributed throughout the epithelial layer produce trefoil factors and mucins, which make up the protective mucous layer. To perform these functions, IECs are armed with a number of sensing mechanisms, including pattern recognition receptors such as Toll-like receptors, nucleotide-binding oligomerization-domain protein-like receptors (NLRs), and cytokine and chemokine receptors (65, 66).

The epithelial response to such a multitude of signals is governed primarily by the balance between the NF-κB and STAT3 signaling pathways. NF-κB signaling in IECs functions as a rheostat controlling apoptosis and proliferation and is central to barrier integrity and host defense. Mendelian disorders suggest that defective NF-κB activation can lead to intestinal inflammation as suggested by defects in *IKBKG* (NEMO), the ubiquitin ligase A20 encoded by *TNFAIP3* (67), and *RELA* haploinsufficiency (68). Cell type–specific deletion or bone marrow chimera experiments confirm that defective NF-κB signaling in epithelial cells is sufficient to confer defects in barrier integrity and subsequent inflammation (68, 69). This has direct consequences since hematopoietic stem cell transplantation can cure susceptibility to infection in *IKBKG*-defective patients but does not cure intestinal inflammation (70).

Leukocytes produce a number of cytokines with functional effects on the epithelium including IL-6, IL-11, and IL-22, which stimulate STAT-3 and promote IEC activation and proliferation. IL-6 signaling on IECs induces YAP/notch signaling that promotes differentiation of absorptive epithelium and contributes to inflammation-driven repair pathways (71). IL-6 is elevated during intestinal inflammation and has been implicated in the pathogenesis of mouse models primarily through its antiapoptotic function on T cells (72). Results of clinical trials in CD with anti-IL-6R blockade show some response (73), but the role of IL-6 in IEC repair may ultimately limit effectiveness (74).

Type-17 lymphocytes including Th17 cells and type 3 innate lymphoid cells (ILC3s) play an important role in host defense at barrier surfaces but are also implicated in IBD pathogenesis. A major component of Th17 and ILC3 biology is the interaction of these cells with the intestinal

epithelium through the production of cytokines IL-22 and IL-17. In the intestine, the IL-22R is restricted primarily to IEC where it activates STAT3 promoting host defense and repair through induction of cell proliferation, mucins, and antimicrobial peptides (75–77). Alongside host defense, recent studies have highlighted the ability of IL-22 to act directly on intestinal stem cells leading to increased repair following damage induced by graft-versus-host disease (GVHD) (78). Consistent with its role in epithelial barrier function and host defense, blockade of the IL-22/IL-22R pathway increases colitis in some mouse models (79, 80). IL-22 binding protein, which acts as an IL-22 antagonist, is increased in IBD patients (81). Thus, therapies that increase IL-22 may be protective in IBD. However, the effects of IL-22 are context dependent since IL-22 can mediate microbe-driven intestinal inflammation and colon cancer (82, 83). Indeed, the finding that IL10RB mutations (encoding a component of the IL-22R) in infantile IBD are corrected by bone marrow transplantation (84) suggests that IL-22 signaling in the epithelium is redundant for intestinal homeostasis. Similarly, autoimmune polyendocrinopathy candidiasis ectodermal dystrophy patients, some of whom develop high-affinity neutralizing anti-IL-22 antibodies, do not develop IBD (85).

The IL-17 receptor is expressed on multiple hematopoietic and nonhematopoietic cells in the intestine (86). A number of leukocytes produce IL-17 including Th17 cells, γδ-T cells, and ILC3s. In IEC, IL-17 signaling predominantly activates NF-κB and promotes host defense to extracellular pathogens through induction of neutrophil attractants and antimicrobial peptides (86). Although IL-17 is pathogenic in some models of colitis (87), it plays a protective role in most studies (88, 89). Recently, acute production of IL-17 by γδ + T cells was shown to be required for correct functioning of IEC tight junctions and barrier integrity (90, 91). In addition, IL-17 synergized with fibroblast growth factor to stimulate repair in an intestinal damage model of colitis (89). These barrier- and repair-promoting functions of IL-17 in the intestine revealed in mouse models may explain why clinical trials of an anti-IL-17A antibody failed in CD (92) despite showing good efficacy in psoriasis (93). Inborn errors of IL-17 immunity in humans manifest in restricted immunodeficiency presenting as mucocutaneous candidiasis (94). These patients do not develop IBD, suggesting the host-protective functions of IL-17 in the intestine may be most significant in the presence of inflammation.

In addition to its antimicrobial activity, defective autophagy likely contributes to disease activity in IEC via goblet cell mucous secretion and Paneth cell activity (95). Epithelial-specific deletion of Atg16l1 shows the impact defective autophagy has on Paneth cells, indicating a threshold model where age-dependent accumulation of IRE1 (96), endoplasmic reticulum stress caused by XBP1 defects (97), and viral infection (98) act as additional susceptibility factors for intestinal inflammation. The context-specific effects of the common human CD-associated variant ATG16L1 T300A are explained by a caspase cleavage site that causes depletion of ATG16L1 during inflammation (99). Given these results, an attractive therapeutic concept is to induce autophagy in patients with genetic defects to restore the defective secretory or antimicrobial activity (100). It needs to be shown whether therapeutically relevant induction of selective autophagy can be achieved despite intrinsic genetic defects in IBD patients.

IL-18 plays a key role in the IEC inflammatory response. Mouse models suggest that epithelial-derived IL-18 regulates colitogenic Th17 cell differentiation as well as intestinal regulatory T cell function (101, 102). Increased amounts of IL-18 were found in IBD patients (103) and in the serum of patients with mutations of NLRC4 who develop infantile enterocolitis and autoinflammation (104). Importantly, blockade of IL-18 as well as IL-1 receptor signaling by Anakinra led to the resolution of otherwise therapy-resistant enterocolitis (105). As such, blockade of IL-18 may be beneficial in subgroups of IBD patients with autoinflammatory mechanisms.

Mesenchymal Cells

Mesenchymal cells are abundant in the intestine where together with extracellular matrix components they constitute the connective tissue underlying the epithelium. Originally regarded as primarily structural, there is accumulating evidence that mesenchymal cells integrate IEC and immune responses in the intestine contributing to host defense, inflammation, and tissue repair (106). By comparison with other cell lineages in the intestine, our understanding of mesenchymal cells is primitive and primarily based on expression of markers. Three major subsets have been identified: fibroblasts, alpha smooth muscle actin (α-SMA)-expressing myofibroblasts, and perivascular pericytes (106). During inflammation, myofibroblasts are activated in response to various inflammatory cytokines. To repair damaged tissue, extracellular matrix components such as collagens are produced. Fibroblasts also act as sentinels in the intestine. Through activation of NOD2, mesenchymal cells produce CCL2, a monocyte chemoattractant that protects against enteric pathogens (107). Pericryptal fibroblasts, which line the intestinal crypts, provide key growth and differentiation factors for intestinal IEC stem cells to contribute to epithelial repair and restitution (108).

As with epithelial barrier function, host-protective functions of mesenchymal cells are a double-edged sword, and in IBD, the constant cycle of tissue inflammation and repair leads to overproduction of extracellular matrix and other common complications such as the development of fibrosis. TNF is directly profibrotic on intestinal myofibroblasts (109), but anti-TNF therapy has limited effects on fibrotic disease, suggesting alternative pathways promote fibrosis in IBD. IL-13 is another cytokine with marked profibrotic properties that acts directly on fibroblasts and intestinal macrophages to induce transforming growth factor beta (TGF-β) and the profibrotic response. Although anti-IL-13 blockade did not induce remission in clinical trials (110, 111), its potential antifibrogenic role has not been assessed in long-term studies.

Mesenchymal cells also contribute to the pathogenic inflammatory response in IBD. Pioneering studies in the Kolias lab showed that mesenchymal cells are the primary target of pathogenic TNF and sufficient for the development of Crohn's-like ileitis in mice (112). Similarly defective NF-κB signaling in a subset of intestinal mesenchymal cells inhibits chemical colitis and inflammation-driven cancer in mice (113). Recent evidence in IBD has shown that intestinal fibroblasts express an activated phenotype with enhanced responsiveness to cytokines (114). Among these, the IL-6 family cytokine Oncostatin M (OSM) and its receptor OSMR are increased in active CD and UC (114). OSM is produced by leukocytes, whereas OSMR is expressed primarily on intestinal mesenchymal cells. Fibroblast populations, in particular, are affected and respond by inducing chemokines and cytokines involved in recruitment and retention of leukocytes. High OSM and OSMR levels correlate with nonresponse to TNF-neutralizing therapy in IBD and OSM neutralization attenuated anti-TNF resistant colitis in mice. These data suggest that OSM-mediated activation of fibroblasts drives the pathogenesis of IBD through a pathway distinct from that of TNF. OSM is thus a potential biomarker and therapeutic target for IBD with particular relevance for anti-TNF-resistant patients.

Innate Barrier of Phagocytes: Crohn's Disease as an Innate Immunodeficiency

Neutrophils, monocytes, and monocyte-derived macrophages are the main phagocytes in the intestine and, thus, constantly respond to translocated or invading bacteria. These cells accumulate during intestinal inflammation and form the histological hallmarks of inflammation: neutrophil-enriched crypt abscesses in UC and granulomas in CD. Traditionally seen as a primary hyperinflammatory disorder, CD in particular has been increasingly recognized to include an immunodeficiency element in its pathogenesis. This is supported by development of CD-like intestinal

inflammation in diseases caused by several phagocyte defects and the identification of defective antimicrobial autophagy in CD (38, 39, 95, 115).

Autophagy, a process of self-digestion, is involved in recycling of intrinsic cellular components. As a component of a cell's intrinsic defense system, autophagy is involved in antimicrobial activity (xenophagy) and defects are particularly associated with CD. Thus, NOD2 signaling, the strongest CD susceptibility factor (116, 117), links ATG16L1 signaling with defective antimicrobial autophagy in dendritic cells, monocyte-derived macrophages, and neutrophils (118–120). Several Mendelian disorders that present with CD-like granulomatous colitis (e.g., XIAP and NPC1 defects) are also associated with defective NOD2–induced autophagy (119). As for autophagy defects in epithelial cells, correcting autophagy is also a potential therapeutic intervention in phagocytes. The mTOR inhibitor rapamycin induces autophagy and bacterial clearance in monocyte-derived dendritic cells with genetic variants of NOD2 in vitro (118), but its potential therapeutic application in CD is limited owing to dose-dependent toxicity (118, 121, 122). Other autophagy-inducing drugs that trigger antibacterial activity in vitro in mouse and human phagocytes with *Atg16l1* and *NPC1* genetic defects include phenothiazines (119, 123).

The hypothesis that CD in particular has an immunodeficiency component can account for the increased inflammasome activation and production of cytokines seen in IBD patients as a secondary consequence of increased intracellular bacteria. Phagocytes mount potent inflammatory responses to translocating phagocytosed bacteria including TNF, IL-23, and IL-1. In addition to the well-recognized roles of anti-TNF and anti-IL-23, secondary inflammasome activation may be critical since mouse models (124, 125) and human studies suggest that blocking IL-1 may have a therapeutic benefit in a setting of autoinflammation in patients with mevalonate kinase deficiency (44), gain-of-function NLRC4 defects (126), or IL-10 receptor defects (125). In summary, genetics and disease mechanisms clearly support the element of immunodeficiency with defective bacterial handling in CD, but the attractive therapeutic potential of stimulated or restored xenophagy needs to be explored and evaluated beyond preclinical studies.

Targeting the IL-23/TH17 Axis

The discovery of IL-23 and Th17 cells represented a step change in our understanding of the immunity of barrier surfaces in health and disease and has driven development of a number of new therapeutic targets for IBD (86, 127). IL-23 is a heterodimeric cytokine that shares the p40 subunit with IL-12 and is produced primarily by activated monocytes, macrophages, and dendritic cells. Its receptor is composed of IL-23R and IL-12RB1 (IL-12RB1 can also pair with IL-12RB2) and is present on T cells and innate lymphoid cells including Th-17 cells, γδ-T cells, invariant T cells, and ILC3s. IL-23 signaling in activated Th17 cells triggers JAK2 and STAT3 signaling, leading to production of signature Th17 cytokines such as IL-17 and IL-22 (127). A key feature of innate and adaptive IL-23-responsive lymphocytes is their expression of the transcription factor RORγt, which is required for IL-23R expression and IL-23-driven production of type 17–associated cytokines (128). IL-12 and IL-23 are increased in CD and UC. However, studies in mouse models of chronic intestinal inflammation highlight the pivotal role of IL-23 and the IL-23R in disease development (129).

IL-23 is not required for the development of Th17 cells but functions to promote their survival and effector function particularly during the inflammatory response (130–132). Th17 cells in the intestine are independent of IL-23 and may contribute to intestinal homeostasis through the tonic production of barrier-promoting cytokines such as IL-17 and IL-22 (133). By contrast, IL-23 drives more pathogenic Th17 cells that induce intestinal inflammation through production of a mixture of cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF) and

IFN-γ that drive myeloid cell activation (134). In addition to promoting inflammatory pathways, IL-23 also antagonizes regulatory pathways through inhibition of the intestinal regulatory T cell response (135, 136).

Study of IL-23-driven innate responses led to the identification of IL-23R-expressing innate lymphoid cells (ILC3s) (137–139). ILC3 populations are present in the intestine, and in mouse models, they can drive intestinal pathology and barrier-promoting host-protective responses through their production of IL-17 and IL-22, indicating context-dependent functions (139, 140). Although ILC3s are increased in the inflamed intestine (141), their functional role in disease pathogenesis has not been established.

Functional studies combined with identification of multiple GWAS hits in the IL-23/Th17 axis have provoked intense interest in targeting this pathway in IBD. Ustekinumab, an anti-IL-12/23p40 antibody, was effective for the treatment of CD (142), and anti-IL-23p19 antibodies have shown similar efficacy (143, 144). Targeting IL-23, rather than IL-17, is a better option because it inhibits Th17-mediated immune pathological pathways while preserving crucial barrier-promoting functions of the host-protective IL-17 response (90, 91). Other strategies to inhibit the IL-23/Th17 axis include targeting the downstream JAK2/STAT3 pathway, although the pleiotropic functions of these pathways in distinct cell types may limit this approach (145). More recently, RORγt antagonists have been developed (146). These show efficacy in mouse models of colitis and inhibit Th17 responses in vitro (147). Interestingly, transient inhibition of RORγt ameliorates colitogenic Th17 responses but spares host-protective ILC3s, suggesting differential requirements for RORγt in Th17 and ILC3 function (147). This all illustrates that the IL23/Th17 axis is a key therapeutic target in IBD and several medications have already been used in the clinic (148).

CD8 Cells: More Than a Biomarker?

In contrast to the clear evidence of a functional contribution of $CD4^+$ T cells to pathogenic and immune-regulatory networks during intestinal inflammation, the important role of $CD8^+$ T cells for intestinal physiology and disease is only emerging (149). Indeed, only a few animal models suggest a pathogenic role for $CD8^+$ T cells (150). However, $CD8^+$ T cells are differentially activated in patients with IBD and might serve as a biomarker since a $CD8^+$ T cell exhaustion transcriptional profile predicts clinical outcome (151, 152).

B Cells in Intestinal Inflammation

The pathogenic role of B cells is another matter of debate. IgA coating of bacteria is associated with intestinal inflammation and can be used to identify colitogenic bacteria (153). The immunoglobulin response might be pathogenic, protective, or a bystander activation reflecting pathogenic T cell responses. Several MD-IBD patients present with agammaglobulinemia (for instance, those cases caused by loss-of-function Bruton kinase variants encoded by *BTK* or loss-of-function PI3Kp85a encoded by *PIK3R1*) and combined variable immunodeficiency (38). Immunoglobulin reconstitution in those patients reduces pulmonary infection susceptibility but not intestinal inflammation, suggesting that IBD is unlikely to be mediated via deficiency of immunoglobulins.

In most IBD patients, the presence of serum antibodies (such as pANCA, anti–*Saccharomyces cerevisiae* antibodies, anti-OmpC, and antiflagellin) is likely a bystander response, although occurrence of multiple antibodies might predict a more severe form of IBD and its complications (154). An extreme example of antibody-mediated autoimmune skin and intestinal epithelial damage associated with intestinal inflammation is epidermolysis bullosa acquisita (155). These patients present with antibodies against type VII collagen. Antibodies toward type VII collagen are pathogenic since

they can induce epithelial damage in animal models (156). It is interesting to note that antibodies against GM-CSF are a marker of aggressive CD (157), and recently a frameshift loss-of-function variant in the GM-CSF receptor (encoded by *CSF2RB*) was associated with increased risk for CD (158). Thus, in subsets of IBD patients, specific autoantibodies might be not just biomarkers but also contributors to pathogenesis.

Promoting Immunoregulatory Pathways

In addition to increased proinflammatory activity, loss of regulatory elements promotes intestinal inflammation. As mentioned above, there is conclusive evidence that IL-10 signaling plays a key role in maintaining intestinal homeostasis. Mouse models with targeted defects in *Il10* (159) and *Il10rb* (160) develop spontaneous colitis. The exceptional role of IL-10 signaling is evident from the development of infantile-onset IBD in children with loss-of-function defects in *IL10* or its receptor (40, 41, 161). Common polymorphisms in the locus of *IL10* (162) and in *IL10RB* have been associated with IBD (7) but were not resolved to a single variant and gene (17).

IL-10 is produced by several cell types including regulatory T cells, B cells, monocytes, and macrophages (163). IL-10 produced by macrophages is dispensable for gut homeostasis, but IL-10 receptor signaling in macrophages is essential since targeted deletion of IL-10R signaling in macrophages results in spontaneous development of colitis (164, 165). IL-10 controls the pro-inflammatory lipopolysaccharide-driven cytokine response via STAT3 signaling (163). In addition, the immunological functions of IL-10 in macrophages are associated with adaptation in cellular metabolism since IL-10 controls macrophage glucose uptake, glycolysis, oxidative phosphorylation, mammalian target of rapamycin (mTOR) signaling, and mitophagy (166).

Although IL-10 controls a potent anti-inflammatory pathway, no direct therapeutic applications to boost IL-10 in IBD patients have reached the clinic. This is partially explained by side effects observed when IL-10 is provided systemically (167, 168). Intestinal delivery of IL-10 via *Lactococcus* has been effective in a mouse model (169) and is safe in humans (170). Medications that target IL-10 to the inflamed intestine may offer efficient delivery of IL-10 to its site of action.

TGF-β is also a negative regulator of intestinal inflammation (171). In IBD there is increased expression of the negative regulator of TGF-β signaling SMAD 7, resulting in impaired TGF-β-mediated control mechanisms (172). These results led to the development of an oral SMAD7 antisense oligonucleotide aiming to block SMAD7 and restore TGF receptor signaling. Although initial clinical trials showed substantial response rates in patients with CD (173), recent interim analysis led to the termination of ongoing trials due to lack of efficacy (174).

The central role of $FOXP3^+$ regulatory T cells in controlling autoimmunity and intestinal inflammation is illustrated by the development of IPEX syndrome in humans (175) and mice with *Foxp3* defects (176). Regulatory T cells control inflammation in mouse models via a number of mechanisms that include secretion of IL-10 and TGF-β, cell-cell interactions via Ctla4, PI3K-Akt-mTOR-Foxo signaling, and other pathways that affect immune metabolism (177, 178). Mouse models suggest that thymic-imprinted and induced regulatory T cells control innate and adaptive immune responses (179). Given that regulatory T cells require IL-2 for their normal functioning, it is interesting that an intronic IBD susceptibility polymorphism in the *IL2RA* locus (17) changes the balance between regulatory T cells and Th17 cells (180), supporting an immune dysregulation element in classical IBD. It needs to be shown whether stimulation of endogenous regulatory T cells via low-dose IL-2 in UC (NCT02200445) or cellular therapy of autologous in vitro–expanded regulatory T cells will be beneficial in classical IBD (181). Restoration of regulatory T cell homeostasis via low-dose IL-2 can prevent intestinal GVHD (182, 183) and shows the feasibility and potential of this approach to control tissue inflammation (184).

MANIPULATION OF INTESTINAL DYSBIOSIS

IBD in humans as well as model systems is associated with reduced diversity of gastrointestinal bacterial communities, termed dysbiosis. The presence of (likely pathogenic) species such as adherent invasive *E. coli* strains and a reduction of (likely beneficial) bacterial community members such as *Faecalibacterium prausnitzii* suggest a shift in the balance between colitogenic and protective bacteria (185, 186). Most mouse models suggest that development of intestinal inflammation depends on the presence of the microbiota. This is not unselective since—depending on host susceptibility—only some bacteria, such as segmented filamentous bacteria or *Helicobacter hepaticus*, drive colitis via induction of Th17 cell responses (187–190). Similar to bacterial dysbiosis in IBD (191), alterations in the fungal microbiota may either be a bystander response or play an as-yet underappreciated role (192). Associated with the reduced diversity of bacterial microbiota, the human virome in IBD patients is substantially altered, in particular, via increased diversity of bacteriophages (193). Further studies are required to determine the functional significance of this finding.

Manipulation of microbiota composition, diversity, and functionality via probiotics, fecal transplantation, prebiotics, or antibiotics may provide an opportunity to target dysbiosis and restore eubiosis. Use of bacterial products such as butyrate or polysaccharides may further modulate immune homeostasis via natural mechanisms. Evidence to support this concept comes from induction or maintenance of remission in UC by probiotic bacteria such as VSL#3 (a mixture of several bacteria that includes *Bifidobacteria*, *Lactobacilli*, and a *Streptococcus thermophilus* strain) or the probiotic strain *E. coli* Nissle 1917 (194). An unresolved question in understanding IBD regards the differences in responses to probiotics between CD and UC.

There is some evidence that recolonization of the gastrointestinal tract via fecal transplantation has a potentially therapeutic role in UC (195). Whereas antibiotics are a risk factor for development of intestinal inflammation—presumably by reducing the intestinal diversity with lasting effects (196)—their role as therapeutics is limited.

In addition to supplementation of bacteria, there is also interest in postbiotics, i.e., bacterial products that exploit evolved pathways that are beneficial to the immune system (186). In mouse models, postbiotics such as polysaccharide A (197) or the histone-deacetylase inhibitor butyrate can stimulate intestinal regulatory T cells (198). In summary, mouse models and human data suggest that intestinal microbiota are a key driver of intestinal inflammation. Multiple bacteria cause pathogenicity depending on metatranscriptomic similarities, the degree of invasiveness, and individual host susceptibility.

Stem Cell Transplantation: Restarting the Immune System

One extreme conceptual approach to treat IBD is replacement of entire cellular compartments via hematopoietic, mesenchymal, or bowel transplantation. Mendelian disorders with immunodeficiency and IBD confirm that this approach can be highly effective. In several monogenic immunodeficiencies such as IL-10 signaling defects, allogenic HSCT is the current standard of care (84). Patient selection is key for this procedure since MD-IBD patients with epithelial defects, such as those caused by mutated *EPCAM* (199, 200) or *TTC7A* (201, 202), are unlikely to benefit from an HSCT approach.

Due to procedure-related adverse effects, there is no direct translation to polygenic IBD. In patients with refractory CD who received autologous HSCT, no significant improvement as indicated by sustained disease remission at 1 year was observed, although there was significant toxicity (203). It is currently not clear whether lack of HSCT efficacy is due to the autologous

approach, i.e., replenishment of patient-derived hematopoietic cells (in particular with remaining innate immune defects), or due to a substantial epithelial component in CD patients that cannot be corrected. An alternative approach might be mesenchymal stem cell transplantation to modify the stromal microenvironment (204, 205).

Gene Therapy: Correcting the Causative Defect

Despite the significant advances in therapeutic concepts reviewed here, many current and future treatments do not reverse the cause of the disease. In MD-IBD where single-gene defects cause intestinal immunopathology, gene therapy offers the possibility of correcting the gene defect in patient-derived cells. The feasibility of gene therapy for improving and curing immunodeficiency and colitis has been shown in Wiskott-Aldrich syndrome (206, 207). Genotoxicity was observed in the early-generation vectors (207), but novel vectors or CRISPR/Cas9-mediated gene transfer and base editing may offer potentially safer approaches in the future.

PREVENTION OF INFLAMMATORY BOWEL DISEASE

The rising incidence and prevalence of IBD in the last half-century in Europe and Western America and the increasing numbers of patients in developing countries suggest that environmental factors play a major role in IBD pathogenesis. Multiple factors that affect IBD susceptibility, including breastfeeding, antibiotic exposure in infancy, smoking, major life stressors, and diet, have been identified (196). Many of these environmental factors reduce the diversity of intestinal microbiota and may have long-term effects via priming and imprinting of the immune system at an early age. To stop or even revert the rise in IBD incidence, mechanistic understanding of those risk factors is needed to inform population-based preventive interventions. In light of the extreme numbers needed to treat and the decades of follow-up required to see robust effects, prospective controlled interventional studies are difficult to perform. However, it will be informative to see whether changes in lifestyle such as the substantial reduction in the prevalence of smoking in many countries in the last 30 years (208) will reduce the incidence and prevalence of IBD and affect the ratio between CD and UC. Ultimately, prevention of IBD might become a rational complementary strategy to therapeutic interventions.

SUMMARY

Emerging evidence suggests that a state of metagenome instability, immune dysregulation, and defective mucosal barrier function is the underlying cause of MD-IBD as well as classical polygenic IBD. In subtypes of Mendelian disorders, correcting the underlying functional pathway using targeted therapies is feasible, and genomics can inform personalized medicine. In classical IBD with multifactorial pathogenesis, multiple functional perturbations of intestinal checkpoints lead to similar histological endpoints. As a consequence, restoring barrier function and antimicrobial autophagy may be a complementary strategy to inhibition of inflammatory cytokines and targeting of immune cell subsets.

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