Published in final edited form as:

Inflamm Bowel Dis. 2014 May ; 20(5): 956–966. doi:10.1097/01.MIB.0000442923.85569.68.

Could a Swimming Creature Inform Us on Intestinal Diseases? Lessons from Zebrafish

Ye Yang, PhD[*], **Sarah Tomkovich, BS**[*,†], and **Christian Jobin, PhD**[*,‡]

[*]Department of Medicine, University of Florida, Gainesville, Florida

[†]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[‡]Department of Infectious Diseases and Pathology, University of Florida, Gainesville, Florida

Abstract

Understanding a complex pathology such as inflammatory bowel disease, where host genetics (innate and adaptive immunity, barrier function) and environmental factors (microbes, diet, and stress) interact together to influence disease onset and severity, requires multipronged approaches to model these numerous variables. Researchers have typically relied on preclinical models of mouse and rat origin to push the boundary of knowledge further. However, incorporation of novel vertebrate models may contribute to new knowledge on specific aspects of intestinal homeostasis. An emerging literature has seen the use of zebrafish as a novel animal system to study key aspects of host–microbe interactions in the intestine. In this review, we briefly introduce components of host–microbiota interplay in the developing zebrafish intestine and summarize key lessons learned from this animal system; review important chemically induced and genetically engineered zebrafish models of intestinal immune disorders; and discuss perspectives and limitations of the zebrafish model system.

Keywords

Intestinal epithelial cell; microbiota; host-microbe interactions; homeostasis; intestine

Inflammatory bowel diseases (IBDs), such as Crohn's disease and ulcerative colitis, affects millions of people in the western world and shows rapidly increasing incidence and prevalence in developing countries in Asia.[1–7] The etiology of human IBD is still unclear, but the pathology has been recognized as multifactorial, involving intricate interplay between the immune system, intestinal microbes, genetic factors, and environment.[8]

The role of microbial entities, especially commensal bacteria, in disease development has taken center stage in IBD research. Using next-generation sequencing of microbial genes, researchers have achieved unprecedented resolution of the intestinal microbiota, and

Reprints: Christian Jobin, PhD, Department of Infectious Diseases and Pathology, College of Medicine, University of Florida, Gainesville, Florida, 2015 SW 16th Avenue, V3-151, PO Box 110880, Gainesville, FL 32611-0882 (Christian.Jobin@medicine.ufl.edu).

The authors have no conflicts of interest to disclose.

numerous studies have revealed marked differences in microbial composition between IBD patients and healthy subjects. [9–11] In general, the IBD-associated intestinal microbiota displays reduced species richness and diversity, and lower temporal stability, with particular bacterial taxa being enriched (e.g., *Enterobacteriaceae*) or depleted (e.g., *Lachnospiraceae*). In addition, great progresses have been made on the host genetic side with the identification of more than 163 IBD susceptibility loci in the human genome, many of them associated with the innate and adaptive immunity, bacterial host response, and autophagy.[12] There is also a growing appreciation of environmental factors as important contributors to IBD. Correlations between IBD and diet, medication, smoking, lower plasma vitamin D, psychosocial stress, etc., are being actively explored.[13–15]

A number of animal IBD models, mostly of murine origin, have been developed over the years to functionally address the importance of these various microbial, genetic, and environmental factors.[16] These models have provided key information regarding the role of epithelial barrier function, innate sensors, adaptive immunity, autophagy, microorganisms, and cellular compartment implicated in disease susceptibility and development. Despite the great contribution of murine models to IBD research, a number of limitations such as cost, imaging capacity, and genetic manipulation have hindered progress, and scientists have explored the potential of incorporating other vertebrate systems in their experimental approaches. The introduction of a new model system would need to take into account key aspects of the human intestine, which is the presence of innate and adaptive immunity, epithelial barrier, and a microbiota, all essential contributors to IBD. Moreover, the model system would need to provide a robust and novel way to study host–microbe interactions, as well as to allow manipulation (genetic/pharmacological) of these interplays to gain new insights into disease pathogenesis.

The zebrafish (*Danio rerio*) has gained popularity among researchers interested in host–microbe interactions in the gut mucosa[17,18] and modeling human immune disorders such as IBD.[19] This lower vertebrate has many advantages over higher mammal systems: low cost to produce and maintain in laboratory conditions, small size, rapid development, high fecundity, and optical transparency at embryonic and larval stages (even at adulthood for certain zebrafish lines[20]). Other important properties that contribute to the rapidly growing popularity of zebrafish include sophisticated genetic manipulation techniques to carry out forward and reverse genetics,[21,22] delayed maturation of adaptive immunity[23,24] enabling focused investigation of innate immunity, and an aqueous living environment allowing easy temporal control over microbial and chemical interventions. Finally, the presence of a diverse microbiota and the ability to manipulate the microbial ecosystem through germ-free (GF) derivation and gnotobiotic techniques in zebrafish clearly represent a unique opportunity to study key aspects of host–microbe interactions and evaluate functional impacts on health and diseases.

HOST–MICROBE INTERACTIONS IN THE DEVELOPING ZEBRAFISH INTESTINE

The zebrafish Gut, Intestinal Microbiota, and Immunology

The zebrafish digestive system undergoes rapid development with morphogenesis initiated at approximately 18 hours after fertilization.[25–27] At 2 to 3 days post fertilization (dpf), the larvae hatch and a continuous gut tube is formed. Soon after, the mouth and vent open, exposing the intestinal lumen to the environment, including microorganisms. By 5 dpf, the digestive tract supports feeding and digestion, and many digestive organs, such as the intestine, liver, gall bladder, and pancreas, functionally resemble their counterparts in mammals.

The adult zebrafish intestine is highly analogous to that of mammals.[26,28–30] It consists of 1 long tube which folds twice in the abdominal cavity. Along the rostrocaudal axis, the intestine can be arbitrarily divided into 3 portions based on morphology: the intestinal bulb, the mid-intestine, and the caudal intestine. Gene expression analysis suggests functional segmentation of a small and large intestines, similar to that found in mammals.[29] The gut lumenal surface is covered by a single layer epithelium that forms irregular ridge structures (Fig. 1). Three differentiated cell types have been identified in the gut epithelium: absorptive enterocytes, mucus-producing goblet cells, and enteroendocrine cells. Underneath the gut epithelium is the lamina propria, which harbors diverse mononuclear cells (monocytes, macrophages, and neutrophils) involved in gut immunity.[30] Noticeably, compared with the mammalian intestine, the zebrafish gut lacks crypts and submucosal glands.[29] Also, no Paneth cells have been identified[25] in the zebrafish gut epithelium, although high intestinal expression of several β-defensins is detected.[31]

The initial contact between the zebrafish larva and environmental microbes occurs at hatching, and thereafter the zebrafish gut microbiota forms. Next-generation sequencing of bacterial 16 rRNA genes from laboratory-reared zebrafish intestines revealed abundant presence of the bacterial phyla Proteobacteria, Firmicutes, and Bacteroidetes, which are also the dominant members of the human and mouse gut microbiota,[32,33] although at a different proportion. Other bacterial divisions like Verrucomicrobia and Actinobacteria are also shared among the teleost, mouse, and human. Longitudinal studies following the gut microbiota changes during development show that the bacterial community becomes more divergent as the fish grows.[34] A recent pilot investigation indicates that the intestinal bulb appears to have higher bacterial diversity than the caudal intestine, with Gammaproteobacteria increasing while Bacilli decreasing along the rostro–caudal axis.[35] Interestingly, Gammaproteobacteria are found to be an important bacterial group in intestinal disorders, especially IBD.[36]

Remarkable similarities in the gut microbiota composition have been reported between laboratory-reared zebrafish and wild zebrafish, indicative of a core gut microbiota that is selected by the zebrafish from its living environment.[37] Furthermore, gut microbiota transplant from the mouse to GF zebrafish showed that the gut bacterial community structure gradually changed from the typical mouse pattern to more zebrafish-like,[32]

suggesting host-specific preference over certain bacterial species within the zebrafish gut. Nevertheless, the zebrafish gut microbiota is still amenable by diet, feeding, and environment,[38–40] similar to the situation observed in mice and humans.

At the center of host–microbe interactions is the immune system. We direct readers to a number of comprehensive reviews covering zebrafish immunology.[30,41–49] In general, the zebrafish immune system closely resembles the mammalian system in both the innate and adaptive branches. Zebrafish have putative orthologs for all the mammalian Toll-like receptors (TLRs), a major class of innate immune receptors recognizing specific microbial molecules. Noticeably, functionally conserved orthologs of the human IBD susceptibility genes *NOD1* and *NOD2*[12] are also found in the zebrafish.[50] All the mammalian innate immune cell types, including macrophages, neutrophils, and dendritic cells, have been identified in the zebrafish. Major innate immune signaling pathways (e.g., the MYD88-dependent pathway[51]) and effector mechanisms (e.g., activation of NF-κB[52]) are highly conserved between zebrafish and mammals. Zebrafish also possess specialized adaptive immune cells like B and T cells, which are functionally comparable to those of mammals.

However, the zebrafish immune system has some unique features when compared with the mammalian system. The zebrafish genome has a significant proportion of duplicated genes,[53] indicating sequence homologs of mammalian immune genes may not perform conserved functions in the zebrafish. Indeed, zebrafish *tlr4* genes were found not responsive to bacterial lipopolysaccharide (LPS), the ligand for the mammalian TLR4.[54,55] Anatomically, zebrafish do not have lymph nodes, and the fish intestine lacks Peyer's patches. The zebrafish adaptive immunity also differs from that of mammals in terms of sites of T-cell and B-cell maturation and antibody subtypes.[30] For example, B cells in adult zebrafish are generated in the kidney, whereas those in mammals develop in the bone marrow. Most noticeably, zebrafish adaptive immunity is not fully functional until around 4 weeks after fertilization.[23] This distinctive feature, however, makes the zebrafish larva a unique model to study the innate immunity in health and diseases without the interference of adaptive immunity.

Host–Microbe Interactions at Homeostasis

Myriad studies from humans and mice have established the central role of the commensal gut microbiota in modulating host tissue development,[56] metabolism,[57] and immunity.[58] In contrast, the functional implications of host–microbe interactions in the zebrafish gut have only begun to be investigated. Yet, already a number of important findings have been made with this lower vertebrate system, providing novel perspectives and areas of research regarding the relationship forged between the host and its gut microbiota.

Among the contributing events leading to increased interest in using the zebrafish for host–bacterial interaction studies is the development of gnotobiotic techniques.[33,59] Rawls et al[33] first described generation of GF zebrafish by in vitro fertilization of eggs with sperms, both manually collected from adult fish. GF zebrafish larvae have impaired intestinal epithelial renewal and aberrant enterocyte morphology, hinting to a key role of the microbiota in gut tissue maturation. Gene transcription profiling of GF, CONV (GF fish subsequently colonized with a microbiota), and CONR (conventionally reared) zebrafish larval guts

reveals that the microbiota regulates over 200 zebrafish genes, of which 54 exhibit conserved expression changes in the mouse model and are widely involved in intestinal epithelial renewal, nutrient metabolism, and innate immune responses. Interestingly, monocolonization of GF zebrafish with the zebrafish gut commensal *Aeromonas hydrophila* but not *Pseudomonas aeruginosa* restores the level of the innate immune gene *c3* (complement component 3), whereas expression of the metabolism gene fi*af* (fasting-induced adipose factor) is only regulated by *P. aeruginosa* but not *A. hydrophila* monocolonization. Therefore, specific bacterial species induce particular host responses in the zebrafish, a phenomenon that is also evident in the mouse.[60] Establishing the mechanism(s) by which microbial entities selectively trigger host responses would lead to a better understanding of the dialog taking place between the host and the microbiota.

Subsequently, Bates et al[59] developed a protocol to generate GF zebrafish from naturally fertilized eggs and demonstrated that commensal microbes are required for normal gut development. Compared with age-matched CONR/CONV zebrafish larvae, GF larvae have lower ALPI (intestinal alkaline phosphatase) activity and abnormal intestinal distribution of glycoconjugates (GalNAcα3NAc and Galα1,3Gal), markers for gut epithelium maturation.[61–63] GF larval intestines contain fewer goblet cells and enteroendocrine cells. Also, gut functions, such as protein macromolecule intake and peristalsis, are impaired in GF larvae. Noticeably, heat-killed bacteria or LPS restored ALPI activity but not normal distribution of Galα1,3Gal in GF zebrafish, indicating distinct bacterial signals promote various aspects of host intestine development.

Further investigation, led by Cheesman et al,[64] sheds light on the mechanisms by which the microbiota promotes intestinal epithelial renewal during development. The Myd88 (myeloid differentiation primary response gene 88)-mediated innate immune signaling is required because knockdown of *myd88* significantly reduced intestinal epithelial cell proliferation in CONR zebrafish. A similar role for MYD88 has also been assigned in the murine intestine after infection or injury,[65–67] suggesting an evolutionary conserved function. Commensal microbes or *Aeromonas veronii* alone also stabilizes intracellular β-catenin in the larval intestine and therefore enhances Wnt signaling, a major pathway that stimulates cell proliferation.[68] Investigation into interactions between the microbial and Wnt signaling reveal that the zebrafish gut microbiota promotes intestinal epithelial cell renewal, at least in part, by upregulating Wnt signaling downstream of axin1 (a component of β-catenin degradation complex) and upstream of tcf4 (a transcription factor activated by Wnt signaling).

Another important aspect of the host–microbe interaction is reflected by the reciprocal regulation of the commensal gut microbiota and host metabolism.[57] Using fluorescently labeled fatty acid (FA) analogs, combined with live in vivo imaging, researchers were able to monitor FA absorption in zebrafish larvae in real time,[38,69,70] highlighting the unparalleled power of the zebrafish system over conventional murine models. After incubating zebrafish with fluorescent FA analogs, Semova et al[38] observed that CONV larvae had more and larger lipid droplets (LDs) in the intestinal epithelium compared with GF larvae and had intensified fluorescence signal in the liver, suggesting that the gut microbiota facilitates FA absorption and metabolism. A comparison of fed versus starved

CONV zebrafish revealed the gut-specific enrichment of the Firmicutes phylum on feeding. Importantly, monocolonization of GF zebrafish with the Firmicutes strain *Exiguobacterium* sp. was able to upregulate LD formation in enterocytes, suggesting that Firmicutes contribute to the microbiota-mediated intestinal FA absorption. Together, this study demonstrates the important role of specific microbial entities in regulating host energy balance.[38]

Among the zebrafish genes that are differentially regulated by the presence of commensal microbes, many are putatively involved in innate immune responses.[33,52] By performing whole animal imaging on the NF-κB reporter zebrafish, Kanther et al[52] examined spatial and temporal activation of NF-κB (nuclear factor κ-light-chain enhancer of activated B cells) by microbial colonization in zebrafish larvae. The microbiota was found both necessary and sufficient to initiate NF-κB activation in the digestive tract at around 6 dpf. Noticeably, commensal microbes also upregulated NF-κB in extraintestinal tissues, suggesting a host response at distant sites.

Another study, led by Galindo-Villegas et al,[71] offered further insights into the functional implications of the commensal microbiota-mediated immune modulation in developing vertebrates. Commensal microbes, recognized mainly through the Myd88 signaling pathway in newly hatched zebrafish larvae, strongly induce proinflammatory effectors (*il-1β*, *tnf-α*, etc.), chemokines (*il-8*, *il-8-like*, and *ccl-c25ab*), and antiviral mediators such as *ifnΦ3* (interferon Φ3). Colonization by commensals primes neutrophils for recruitment and activation in response to mechanical injuries and protects fish larvae against spring viremia of carp virus infection. Intriguingly, increased *il-1β* expression was also observed in GF zebrafish after hatching, albeit at much lower levels than in CONR fish, suggesting that additional factors besides microbe-induced Myd88 signaling orchestrate the induction of immune genes.

Although conferring numerous benefits to the host, the gut microbiota also provides a continuous source of antigens and toxins that have the potential to provoke host inflammatory responses. It is not clearly understood how the host gut maintains immune tolerance to commensal microbes. The zebrafish system was key in unraveling one of the underlying mechanisms.[72] Bates et al[72] observed that LPS stimulation or gram-negative bacterial colonization induced ALPI in the larval zebrafish intestine through Myd88 signaling. Conversely, ALPI inhibits LPS-induced innate immune activation, thereby detoxifying LPS and preventing excessive host innate responses to gram-negative commensals. The discovery of the zebrafish ALPI as an in situ peacemaker between the host immunity and gut microbiota has spurred a series of studies investigating the immunoregulatory role of ALPI in other organisms and its beneficial effects for treating inflammation-related intestinal injuries.[73]

The zebrafish has been widely used for studying functional mechanisms of probiotics in animal development and reproduction. [74] Recently, Rendueles et al[75] described a zebrafish oro-intestinal pathogen infection model and revealed distinct protection mechanisms of different probiotic strains. In this model, 6 dpf GF zebrafish larvae were immersed in water containing the channel catfish pathogen *Edwardsiella ictaluri* for 6 hours and were then

transferred and incubated under sterile conditions. *E. ictaluri* was found to colonize in the gut and head of GF zebrafish larvae, induce proinflammatory and anti-inflammatory cytokines (*tnf-α, il-1β, il-22,* and *il-10*), and lead to strong neutrophil recruitment to the perioral region and high mortality. Preincubating GF zebrafish larvae with *Vibrio parahaemolyticus* or 2 *Escherichia coli* strains protected animal against *E. ictaluri*-induced mortality. Interestingly, although *V. parahaemolyticus* monocolonization upregulated cytokine gene expression and inhibited *E. ictaluri*-induced neutrophil redistribution, incubation with *E. coli* did not elicit obvious inflammatory responses and had little effect on cytokine induction and neutrophil redistribution on *E. ictaluri* infection. Further investigation revealed that *V. parahaemolyticus* likely functioned by directly inhibiting *E. ictaluri* growth, whereas the *E. coli* strains protected larvae against *E. ictaluri* infection by presenting adhesion molecules such as F pili to promote intestinal adherence and colonization of *E. coli* and thereby exclusion of *E. ictaluri*. Together, this work highlights the potential of using the zebrafish system to delineate roles of probiotic bacteria in intestinal homeostasis and pathology.

Overall, the highly conserved gut biology and immunology system present in zebrafish suggest a potential use for this vertebrate animal in research related to intestinal immune disorders.

ZEBRAFISH MODELS OF INTESTINAL IMMUNE DISORDERS

A number of zebrafish models mimicking some aspects of human inflammatory disorders have been recently developed. This section describes and discusses key findings made within these models, which are categorized into 2 groups: the chemically induced and the genetically engineered models (Table 1).

Chemically Induced Models of Intestinal Immune Disorders

The first chemically induced enterocolitis model developed in the zebrafish system used the hapten oxazolone (4-ethoxymethylene-2-phenyl 2 oxazolin-5-one). Brugman et al[76] reported that intrarectal administration of 0.2% oxazolone in 50% ethanol induced acute enterocolitis in adult zebrafish, which is manifested by severe thickening of the bowel wall, disruption of the intestinal fold structure, depletion of goblet cells, and infiltration of neutrophils and eosinophils. Colitis was evident at 5 hours after oxazolone injection and persisted for a week, with the most pronounced phenotype in the posterior mid-intestine. At the molecular level, oxazolone upregulated expression of the proinflammatory cytokines *il-1β* and *tnf-α* as well as the anti-inflammatory cytokine *il-10* in the intestine, all highly relevant molecules to human colitis.

An interesting finding from this model is that the composition of the intestinal microbiota influences disease susceptibility, [76] a phenomenon also observed in mice.[77] Oxazolone causes enterocolitis only in zebrafish maintained under stand-alone tank conditions but not in those under continuous flow tank conditions. Subsequent analysis shows that fish maintained in stand-alone tanks have a significantly higher load of intestinal bacteria but a much smaller proportion of the phylum Fusobacteria in their microbiota. Remarkably, pretreating the “dirty” fish with the antibiotic vancomycin, which targets gram-positive

bacteria, mitigated oxazolone-induced colitis. In contrast, pretreatment with colistin sulfate, targeting gram-negative bacteria, albeit causing a more dramatic reduction of the intestinal bacterial load than vancomycin, failed to protect fish against the colitis. Further work would be necessary to address the role of specific bacterial species in this model.

Oxazolone-induced colitis appears mainly driven by neutrophils in the zebrafish as vancomycin but not colistin significantly reduced neutrophil infiltration.[76] Interestingly, the microbiota influences neutrophil location and activity,[78] and it would be important to investigate whether this microbial effect on neutrophils influences colitis development. As opposed, colitis in the murine oxazolone model is mainly mediated by natural killer cells.[79] Nevertheless, the oxazolone-induced zebrafish enterocolitis, in large part, phenotypically mirrors the mouse model.[80] The central role of the microbiota in enterocolitis development is well established by studies from humans and mouse IBD models.[81] Vancomycin was also reported to attenuate colitis in the *Il-10*$^{-/-}$ mouse model.[77] The zebrafish oxazolone colitis model recapitulated these observations, proving the feasibility of using zebrafish to model host–microbe interactions in intestinal inflammation.

Another model of intestinal inflammation consists of immersing the zebrafish larvae in a medium containing the haptenizing agent trinitrobenzene sulfonic acid (TNBS).[82–86] Fleming et al[83] reported that 75 μg/mL TNBS caused profound intestine-specific pathological changes in zebrafish larvae: dilated gut lumen, smoothened lining of the gut, compromised gut barrier, and increased the goblet cell population in the mid-intestine and posterior intestine regions. In comparison, Oehlers et al[85] showed that zebrafish larvae exposed to 75 μg/mL TNBS displayed widespread skin damage but no gross change in intestinal cell morphology or the number of goblet cells. The differential sensitivity of zebrafish to TNBS dosage could be related to husbandry conditions between these facilities. A closer look at the microbiota composition in relation to TNBS sensitivity could help address this possibility. At a concentration of 50 μg/mL, TNBS exposure leads to shortening of the larval mid-intestine, disruption of the intestinal vasculature,[85] and liver discoloration.[86] The low-dose TNBS treatment also resulted in leukocyte enrichment and recruitment from the caudal hematopoietic tissue to the intestine and epidermis,[85,86] enhanced global cell proliferation,[85] and excessive nitric oxide production in the cleithrum and notochord.[86]

Despite some discrepancies, TNBS exposure consistently induces intestinal inflammation and impairs gut functions in zebrafish larvae, reminiscent of higher vertebrate systems.[16] The intestinal inflammation is marked by induction of proinflammatory cytokines (e.g., *tnf-α* and *il-1β*), the protease mmp9, and leukocytosis.[83–85] Gut function disruption is reflected by loss of peristalsis[83] and altered lipid metabolism.[85] The TNBS-induced zebrafish enterocolitis also depends on microbiota-derived signals, as adding broad-spectrum antibiotics before TNBS into the fish medium enhanced fish survival and inhibited expression of proinflammatory cytokines.[85] Interestingly, knockdown of *myd88* rendered zebrafish larvae more susceptible to TNBS, suggesting a protective function for this gene. A similar *Myd88* protective function was also observed in mice exposed to dextran sodium sulfate (DSS).[87]

The zebrafish TNBS enterocolitis model proved useful for evaluating the therapeutic effect of compounds. The antiinflammatory drugs prednisolone and 5-ASA were shown to prevent TNBS-induced intestinal histopathological changes, proinflammatory cytokine induction, and intestinal recruitment of leukocytes in the zebrafish, but only prednisolone was effective for treating the TNBS colitis.[83,85] Nitric oxide synthase (NOS) inhibitors could also rescue the zebrafish TNBS enterocolitis,[83] consistent with observations made in mammalian systems.[88,89] However, thalidomide and parthenolide, which are clinically used for IBD treatment, showed little effect on TNBS-induced zebrafish colitis.[83]

The TNBS zebrafish colitis model also provides a system to investigate inflammation-related factors and signaling pathways that are difficult to explore within other animals. For example, human CXCL8 (IL-8) plays an important role in regulating neutrophil chemotaxis, but absence of murine *Cxcl8* has made functional investigation difficult to perform.[90] Interestingly, TNBS exposure dramatically enhances *cxcl8* expression in the zebrafish larval intestine,[84] suggesting that this system could be useful for studying Cxcl8 function in intestinal inflammation. Heat shock proteins (HSPs) are suggested to ameliorate mucosal damage in IBD,[91] and the mammalian HSP gene HSP70 is highly expressed in the intestinal epithelia on intestinal injury in humans. [92–94] Of interest, TNBS exposure upregulates expression of zebrafish *hsp70* and HSP110 family gene *hspa4a* and *hspa4b*.[82] The physiological importance of HSPs in zebrafish intestinal homeostasis is unclear and would require genetic manipulation.

DSS is another chemical model widely used by the research community to study intestinal inflammation in both mice and rats.[16] Recently, Oehlers et al[86] reported that DSS immersion resulted in enterocolitis in the zebrafish larvae. DSS was added to the larva medium at 3 dpf, and disease manifestations, such as liver discoloration, neutrophil influx into the intestine and epidermis, global cell proliferation reduction, and upregulation of proinflammatory genes (i.e., *tnf-α*, *il*-1β, *il*-8, *ccl20*, and *mmp9*), were evident at 6 dpf. Remarkably, DSS exposure led to strong accumulation of acidic mucins in the intestinal bulb, although no apparent change in goblet cell number was observed.

The striking mucin phenotype associated with DSS-induced zebrafish larval colitis is in great contrast to the murine DSS colitis models, where mucin is typically depleted in the vicinity of inflamed tissue.[95,96] The discrepancy could be because of the deleterious effects of DSS on murine but not zebrafish gut epithelial cells. Indeed, the goblet cell population is preserved in zebrafish larvae after DSS exposure, which likely sustains mucin production. The enhanced mucin retention could provide additional protection to the larval gut epithelium, explaining the lack of severe tissue damage in zebrafish larvae after DSS treatment. In agreement with this observation, DSS pretreatment protected the zebrafish from TNBS-induced inflammation and animal mortality.[86] The DSS-induced mucin enrichment in the intestinal bulb is microbiota dependent because administration of broad-spectrum antibiotics reversed the phenotype.[86]

Because of the distinct mucin phenotype, the zebrafish DSS colitis model provides an ideal system to study mucin regulations by various agents. For example, Oehlers et al[86] demonstrated that retinoic acid suppressed both basal and DSS-induced mucin production in

the zebrafish intestine and therefore rendered the animal more susceptible to chemically induced enterocolitis.

Glafenine is a clinically used nonsteroidal anti-inflammatory drug known to cause gastrointestinal damage in both humans and mice.[97,98] Goldsmith et al[99] reported that administration of glafenine to 5 dpf zebrafish larvae rapidly induced (within hours) endoplasmic reticulum (ER) stress-mediated intestinal injury. The injury was readily perceived by the formation of a visible “tube” in the intestinal lumen, which is composed of sloughing epithelial cells resulted from extensive cell apoptosis. Interestingly, the gut epithelial barrier remained intact after glafenine exposure. Ultrastructure characterization using electron microscopy revealed signs of ER stress and halted cell stress responses in the gut epithelium. Gene expression analysis revealed blockage of the unfolded protein response (UPR) signaling, indicative of impaired cell stress resolution.

Defects in ER stress response and autophagy lead to inflammation in the intestine, thereby contributing to IBD pathogenesis.[100] In this regard, the zebrafish glafenine intestinal injury model provides a unique system to investigate ER stress response and intestinal pathogenesis. The singular appearance and the rapid induction of the “tube” structure in the larval gut lumen make the glafenine model useful for carrying out compound screens to identify cell apoptosis inhibitors, as demonstrated by Goldsmith et al.[99] The investigators discovered that the μ-opioid receptor agonist DALDA markedly reduced the “tube” formation in glafenine-treated zebrafish larvae, suggesting that the μ-opioid receptor signaling pathway regulates apoptosis. Further studies showed that DALDA helped ER stress resolution by activating UPR signaling. In line with the finding, DALDA was also found to protect mice against DSS-induced intestinal injury.[101]

Along with the various chemically induced models, a growing number of zebrafish genetic intestinal immune disorder models are being developed to address roles of specific genes in controlling host–microbe interactions.

Genetic Models of Intestinal Immune Disorders

In mammals, MYD88 is an adaptor protein used in the signaling pathways of all TLRs except TLR3. Although it is not known which zebrafish Tlrs signal through Myd88, proinflammatory gene expression in response to LPS, flagellin, and bacterial infection is Myd88 dependent.[51] Moreover, *Salmonella enterica* clearance and expression of the proinflammatory genes *mmp9*, *il-1b*, and *irak2* are impaired in *myd88* morpholino (MO) knockdown zebrafish.[102,103] Furthermore, *myd88* mutants are more susceptible to acute (*Edwardsiella tarda* and *Salmonella typhimurium*) and chronic (*Mycobacterium marinum*) bacterial infections and have impaired innate immune transcription factors (NF-κB, AP-1) and proinflammatory gene expression (*il1-b*, *mmp9*).[51] The defects in innate immunity and increased susceptibility to bacterial infection in both *myd88*-MO and *myd88* mutant zebrafish are consistent with phenotypes reported in *Myd88*$^{-/-}$ mice.[104–108] The *myd88* mutant zebrafish could be a promising model for studying host–microbe interactions and intestinal inflammation.

In humans, *NOD2* gene mutations have been strongly associated with IBD.[12,109,110] Zebrafish *nod1/2* functions have been assessed by MO knockdown, which revealed that these innate sensors control *S. enterica* infection and expression of dual oxidase to produce bactericidal reactive oxygen species.[50] Mammals with NOD1 or NOD2 deficiencies are also susceptible to bacterial infections,[111–114] indicating functional conservation. However, the ligands for zebrafish Nod1/Nod2 have not been identified.

Zebrafish mutants generated with a retroviral-insertion in the CDP-diacylglycerol-inositol 3 phosphatidyltransferase mutants (*cdipt*^{hi559}) lack phosphoinositide (PI) synthesis and display signs of ER stress in intestinal epithelial cells. These mutant fish showed abnormal intestinal morphology at 5dpf with reduced intestinal epithelial cell proliferation, apoptotic epithelial and goblet cells, inflammation, and bacterial overgrowth,[115] characteristics also observed in human IBD patients and mice deficient in ER stress response components.[116,117] The mechanism by which deficiencies in PI synthesis lead to ER stress is not clear. Nevertheless, phosphoinositide 3-kinase (PI3K) signaling plays an important role in intestinal homeostasis. For example, PI3Kγ has been implicated in the promotion and resolution of DSS and TNBS-induced colitis[118,119] and mice deficient in the PI3Kγ p110δ subunit develop spontaneous colitis.[120]

The *sec13*^{sq198} mutant has a defect in the outer coat of the COPII complex, disrupting protein trafficking from the ER to the Golgi apparatus.[121] The mutant fish show hindered development in the liver, pancreas, and intestine (3–5 dpf), because of reduced proliferation and increased cellular apoptosis. Noticeably, the *sec13*^{sq198} mutant also exhibits ER stress and activated components of the UPR pathway. Whether this mutant develops intestinal inflammation or bacterial overgrowth has not been investigated. Both the *cdipt*^{hi559} and *sec13*^{sq198} mutants will be useful for examining how the UPR and ER stress contribute to intestinal inflammation. Zebrafish have homologs of many of the mammalian autophagy genes such as *Atg16L1*, but their functions and the involvement of *nod1/2* in inducing autophagy have yet to be examined.[122]

A balanced immune response requires the coordinate activation of both proinflammatory and anti-inflammatory cytokines, a network clearly dysregulated in patients with IBD. GWAS studies have identified the *IL-23R* as a susceptibility allele in IBD patients.[12] Adult zebrafish constitutively express *il-23* in their intestine and other tissue types such as gills, muscle, and kidney.[123] Expression of *il-23* can also be induced by LPS administration or *M. marinum* infection.[123] However, the functional role of *il-23* in the zebrafish has not been examined. In mammals, IL-23 promotes TH17 stabilization and expansion, which can lead to excessive activation of the adaptive immunity.[124] The zebrafish could be useful for studying components of the adaptive immunity that contribute to IBD. Such a model would require older zebrafish though, because fish adaptive immunity is not active for the first month.

The IL-10 cytokine plays a critical anti-inflammatory role in the intestine as mice deficient for the gene spontaneously develop IBD[125] and IL-10 receptor mutations in humans are associated with early onset of severe IBD.[126,127] The Il-10 receptor is conserved in zebrafish,[128,129] and the IL-10 cytokine is expressed in the kidney, gills, and gut and is

upregulated on LPS stimulation.[129] A zebrafish *il-10* mutant has been identified as part of the zebrafish mutation project, and this mutant could be a promising model for studying host–microbe interactions and intestinal inflammation.[130]

Expression of the cytokine IL-22 is increased in Crohn's disease patients,[131] and experimental models have highlighted the key role of this molecule in promoting mucosal healing.[132] Recently, *il-22* function was investigated in zebrafish.[133] Zebrafish *il-22* is expressed in the intestine and enriched in the head kidney. The *il-22*-MO zebrafish had normal basal inflammatory responses but showed increased mortality and increased inflammatory gene expression (*il-1β* and *tnf-α*) after *A. hydrophila* infection. Further studies are needed to fully explore the role of this cytokine in maintaining intestinal homeostasis in the zebrafish.

Overall, a number of chemical and zebrafish genetic models are available to address the role of various genes in intestinal disorders and immune response.

PERSPECTIVES AND LIMITATIONS

Zebrafish offers unique opportunity for in-depth study of intestinal host–microbe interactions during homeostasis and in disease states. This system provides visualizing power to determine host cell dynamics in response to either single bacterial species or a complex microbial community at the whole animal level. With its rapid development and ease of genetic manipulation, the zebrafish system can contribute to the functional characterization of various IBD susceptible genes identified by GWAS and immunochip analyses. Microbial manipulation in the zebrafish using gnotobiotic technology is economically more affordable and technically less challenging than in the mouse and can facilitate delineation of novel paradigm implicated in host–microbe interactions in the intestine. The combined power of genetic and microbial manipulation in conjunction with outstanding imaging capacity truly positions the zebrafish system at the forefront of experimental models to study host–microbe interactions. The flexibility of nutrient/chemical interventions (continuously or temporally controlled) in the aqueous environment of zebrafish also represents a valuable advantage for examining the contribution of environmental factors such as diet and medication to intestinal disorders. Moreover, zebrafish could serve as a useful system for performing high-throughput screens of anti-inflammatory compounds or small molecules susceptible to interfere with host–bacterial interactions.

Although the zebrafish represents a powerful tool for gastrointestinal-related research, there are a number of limitations associated with this lower vertebrate. First, substantial differences exist in the habitat and intestinal environment of zebrafish and mammals. Laboratory zebrafish are usually maintained at a temperature of 28°C and the fish intestine is primarily aerobic. These environmental conditions could preclude the study of specific bacterial or microbial communities identified in higher mammals. For example, mouse microbiota transplanted into zebrafish failed to maintain its core structure and instead adopted the recipient profile.[32] Attempts to colonize the zebrafish intestine with members of the human gut microbiota have proved largely unsuccessful because only 2 of 30 human

commensal strains were able to establish residency in the fish intestine.[134] Second, noticeable differences exist between the zebrafish and human immunology, suggesting that immunological observations made in zebrafish may not apply to humans and need to be confirmed in mammalian systems to maximize their translational impact. Finally, technical hurdles are yet to be overcome to fully explore the potentials of zebrafish for studying host–microbe interactions. Most prominently, raising GF zebrafish to adulthood is not possible at present because of lack of adequate nutritional information. Therefore, use of gnotobiotic zebrafish is restricted to the larval stage (till around 8 dpf), making studies on the regulation and function of adaptive immunity in host–microbe interactions impossible. Although protocols have been developed to extend the life span of GF fish to over 1 month, these techniques require labor-intensive work and lack practicality.[75] Another major challenge is the limited availability of fish-specific antibodies and reagents, which hinders characterization of signaling pathways and protein expression involved in host–microbe interactions in the zebrafish. It is likely that these technical limitations will slowly disappear with the growing popularity of the zebrafish system and increasing demand for bioreagents.

One important feature of any model organism is the ability to infer similarity of function with humans. The zebrafish has a well-developed intestine, a diverse microbiota, and a sophisticated immune system, all highly resembling the mammalian counterparts and represent key aspects of human intestinal immune disorders such as IBD. In addition, many observations made in humans and murine models regarding host–microbe interactions during gut homeostasis or at disease states have been recapitulated in zebrafish, indicating the suitability of the fish as a model through which we can better understand human disease control and pathogenesis. The zebrafish provides an excellent opportunity to address questions that are difficult to solve in mammalian systems. Moreover, the translational potential of the zebrafish is clear, especially in the area of high-throughput drug screens. In conclusion, we expect that integration of the zebrafish with other model systems would significantly push forward the boundaries of our knowledge of host–microbe interactions in the intestine, with likely application for inflammatory disorders such as IBD. Exciting advances can be anticipated in the coming years from expanding research efforts using the zebrafish system.

Acknowledgments

Supported by National Institutes of Health RO1 grants DK047700 and DK073338 to C.J.

References

1. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012; 142:46–54. e42. quiz e30. [PubMed: 22001864]
2. Prideaux L, Kamm MA, De Cruz PP, et al. Inflammatory bowel disease in Asia: a systematic review. J Gastroenterol Hepatol. 2012; 27:1266–1280. [PubMed: 22497584]
3. Ng SC, Tang W, Ching JY, et al. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. Gastroenterology. 2013; 145:158–165. e152. [PubMed: 23583432]

4. Levi Z, Shamiss A, Fraser GM, et al. The increasing prevalence of inflammatory bowel diseases among Jewish adolescents and the sociodemographic factors associated with diagnosis. Inflamm Bowel Dis. 2013; 19:1867–1871. [PubMed: 23665967]
5. Chuang CH, Lin SH, Chen CY, et al. Increasing incidence and Lifetime risk of inflammatory bowel disease in Taiwan: a Nationwide study in a low-endemic area 1998–2010. Inflamm Bowel Dis. 2013; 19:2815–2819. [PubMed: 24141711]
6. Wang XQ, Zhang Y, Xu CD, et al. Inflammatory bowel disease in Chinese children: a multicenter analysis over a decade from Shanghai. Inflamm Bowel Dis. 2013; 19:423–428. [PubMed: 23340680]
7. Al-Mofarreh MA, Al-Mofleh IA. Emerging inflammatory bowel disease in saudi outpatients: a report of 693 cases. Saudi J Gastroenterol. 2013; 19:16–22. [PubMed: 23319033]
8. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. Nat Rev Immunol. 2008; 8:458–466. [PubMed: 18500230]
9. Bakhtiar SM, LeBlanc JG, Salvucci E, et al. Implications of the human microbiome in inflammatory bowel diseases. FEMS Microbiol Lett. 2013; 342:10–17. [PubMed: 23431991]
10. Berry D, Reinisch W. Intestinal microbiota: a source of novel biomarkers in inflammatory bowel diseases? Best Pract Res Clin Gastroenterol. 2013; 27:47–58. [PubMed: 23768552]
11. De Cruz P, Prideaux L, Wagner J, et al. Characterization of the gastrointestinal microbiota in health and inflammatory bowel disease. Inflamm Bowel Dis. 2012; 18:372–390. [PubMed: 21604329]
12. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012; 491:119–124. [PubMed: 23128233]
13. Ananthakrishnan AN. Environmental triggers for inflammatory bowel disease. Curr Gastroenterol Rep. 2013; 15:302. [PubMed: 23250702]
14. Bonaz BL, Bernstein CN. Brain-gut interactions in inflammatory bowel disease. Gastroenterology. 2013; 144:36–49. [PubMed: 23063970]
15. Yamamoto T. Nutrition and diet in inflammatory bowel disease. Curr Opin Gastroenterol. 2013; 29:216–221. [PubMed: 23385526]
16. Mizoguchi A. Animal models of inflammatory bowel disease. Prog Mol Biol Transl Sci. 2012; 105:263–320. [PubMed: 22137435]
17. Kanther M, Rawls JF. Host-microbe interactions in the developing zebrafish. Curr Opin Immunol. 2010; 22:10–19. [PubMed: 20153622]
18. Meijer AH, Spaink HP. Host-pathogen interactions made transparent with the zebrafish model. Curr Drug Targets. 2011; 12:1000–1017. [PubMed: 21366518]
19. Goldsmith JR, Jobin C. Think small: zebrafish as a model system of human pathology. J Biomed Biotechnol. 2012; 2012:817341. [PubMed: 22701308]
20. White RM, Sessa A, Burke C, et al. Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell Stem Cell. 2008; 2:183–189. [PubMed: 18371439]
21. Huang P, Zhu Z, Lin S, et al. Reverse genetic approaches in zebrafish. J Genet Genomics. 2012; 39:421–433. [PubMed: 23021542]
22. Lawson ND, Wolfe SA. Forward and reverse genetic approaches for the analysis of vertebrate development in the zebrafish. Dev Cell. 2011; 21:48–64. [PubMed: 21763608]
23. Lam SH, Chua HL, Gong Z, et al. Development and maturation of the immune system in zebrafish, Danio rerio: a gene expression profiling, in situ hybridization and immunological study. Dev Comp Immunol. 2004; 28:9–28. [PubMed: 12962979]
24. Danilova N, Hohman VS, Sacher F, et al. T cells and the thymus in developing zebrafish. Dev Comp Immunol. 2004; 28:755–767. [PubMed: 15043944]
25. Ng AN, de Jong-Curtain TA, Mawdsley DJ, et al. Formation of the digestive system in zebrafish: III. Intestinal epithelium morphogenesis. Dev Biol. 2005; 286:114–135. [PubMed: 16125164]
26. Wallace KN, Akhter S, Smith EM, et al. Intestinal growth and differentiation in zebrafish. Mech Dev. 2005; 122:157–173. [PubMed: 15652704]
27. Wallace KN, Pack M. Unique and conserved aspects of gut development in zebrafish. Dev Biol. 2003; 255:12–29. [PubMed: 12618131]

28. Menke AL, Spitsbergen JM, Wolterbeek AP, et al. Normal anatomy and histology of the adult zebrafish. Toxicol Pathol. 2011; 39:759–775. [PubMed: 21636695]
29. Wang Z, Du J, Lam SH, et al. Morphological and molecular evidence for functional organization along the rostrocaudal axis of the adult zebrafish intestine. BMC Genomics. 2010; 11:392. [PubMed: 20565988]
30. Lieschke GJ, Trede NS. Fish immunology. Curr Biol. 2009; 19:R678–R682. [PubMed: 19706273]
31. Oehlers SH, Flores MV, Chen T, et al. Topographical distribution of antimicrobial genes in the zebrafish intestine. Dev Comp Immunol. 2011; 35:385–391. [PubMed: 21093479]
32. Rawls JF, Mahowald MA, Ley RE, et al. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell. 2006; 127:423–433. [PubMed: 17055441]
33. Rawls JF, Samuel BS, Gordon JI. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. Proc Natl Acad Sci U S A. 2004; 101:4596–4601. [PubMed: 15070763]
34. Yan Q, van der Gast CJ, Yu Y. Bacterial community assembly and turnover within the intestines of developing zebrafish. PloS One. 2012; 7:e30603. [PubMed: 22276219]
35. Lan CC, Love DR. Molecular characterisation of bacterial community structure along the intestinal tract of zebrafish (Danio rerio): a pilot study. ISRN Microbiol. 2012; 2012:590385. [PubMed: 23724326]
36. Mukhopadhya I, Hansen R, El-Omar EM, et al. IBD-what role do Proteobacteria play? Nat Rev Gastroenterol Hepatol. 2012; 9:219–230. [PubMed: 22349170]
37. Roeselers G, Mittge EK, Stephens WZ, et al. Evidence for a core gut microbiota in the zebrafish. ISME J. 2011; 5:1595–1608. [PubMed: 21472014]
38. Semova I, Carten JD, Stombaugh J, et al. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. Cell Host Microbe. 2012; 12:277–288. [PubMed: 22980325]
39. Sullam KE, Essinger SD, Lozupone CA, et al. Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Mol Ecol. 2012; 21:3363–3378. [PubMed: 22486918]
40. Wong S, Rawls JF. Intestinal microbiota composition in fishes is influenced by host ecology and environment. Mol Ecol. 2012; 21:3100–3102. [PubMed: 22916346]
41. Bajoghli B. Evolution and function of chemokine receptors in the immune system of lower vertebrates. Eur J Immunol. 2013; 43:1686–1692. [PubMed: 23719857]
42. Ma D, Wei Y, Liu F. Regulatory mechanisms of thymus and T cell development. Dev Comp Immunol. 2013; 39:91–102. [PubMed: 22227346]
43. Meeker ND, Trede NS. Immunology and zebrafish: spawning new models of human disease. Dev Comp Immunol. 2008; 32:745–757. [PubMed: 18222541]
44. Palti Y. Toll-like receptors in bony fish: from genomics to function. Dev Comp Immunol. 2011; 35:1263–1272. [PubMed: 21414346]
45. Rauta PR, Nayak B, Das S. Immune system and immune responses in fish and their role in comparative immunity study: a model for higher organisms. Immunol Lett. 2012; 148:23–33. [PubMed: 22902399]
46. Rebl A, Goldammer T, Seyfert HM. Toll-like receptor signaling in bony fish. Vet Immunol Immunopathol. 2010; 134:139–150. [PubMed: 19850357]
47. Renshaw SA, Trede NS. A model 450 million years in the making: zebrafish and vertebrate immunity. Dis Model Mech. 2012; 5:38–47. [PubMed: 22228790]
48. van der Vaart M, Spaink HP, Meijer AH. Pathogen recognition and activation of the innate immune response in zebrafish. Adv Hematol. 2012; 2012:159807. [PubMed: 22811714]
49. Xu J, Du L, Wen Z. Myelopoiesis during zebrafish early development. J Genet Genomics. 2012; 39:435–442. [PubMed: 23021543]
50. Oehlers SH, Flores MV, Hall CJ, et al. The inflammatory bowel disease (IBD) susceptibility genes NOD1 and NOD2 have conserved antibacterial roles in zebrafish. Dis Model Mech. 2011; 4:832–841. [PubMed: 21729873]

51. van der Vaart M, van Soest JJ, Spaink HP, et al. Functional analysis of a zebrafish myd88 mutant identifies key transcriptional components of the innate immune system. Dis Model Mech. 2013; 6:841–854. [PubMed: 23471913]
52. Kanther M, Sun X, Muhlbauer M, et al. Microbial colonization induces dynamic temporal and spatial patterns of NF-kappaB activation in the zebrafish digestive tract. Gastroenterology. 2011; 141:197–207. [PubMed: 21439961]
53. Woods IG, Kelly PD, Chu F, et al. A comparative map of the zebrafish genome. Genome Res. 2000; 10:1903–1914. [PubMed: 11116086]
54. Sepulcre MP, Alcaraz-Perez F, Lopez-Munoz A, et al. Evolution of lipopolysaccharide (LPS) recognition and signaling: fish TLR4 does not recognize LPS and negatively regulates NF-kappaB activation. J Immunol. 2009; 182:1836–1845. [PubMed: 19201835]
55. Sullivan C, Charette J, Catchen J, et al. The gene history of zebrafish tlr4a and tlr4b is predictive of their divergent functions. J Immunol. 2009; 183:5896–5908. [PubMed: 19812203]
56. Sommer F, Backhed F. The gut microbiota–masters of host development and physiology. Nat Rev Microbiol. 2013; 11:227–238. [PubMed: 23435359]
57. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012; 489:242–249. [PubMed: 22972297]
58. Kamada N, Seo SU, Chen GY, et al. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol. 2013; 13:321–335. [PubMed: 23618829]
59. Bates JM, Mittge E, Kuhlman J, et al. Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Dev Biol. 2006; 297:374–386. [PubMed: 16781702]
60. Hooper LV, Wong MH, Thelin A, et al. Molecular analysis of commensal host-microbial relationships in the intestine. Science. 2001; 291:881–884. [PubMed: 11157169]
61. Zambonino Infante JL, Cahu CL. Ontogeny of the gastrointestinal tract of marine fish larvae. Comp Biochem Physiol C Toxicol Pharmacol. 2001; 130:477–487. [PubMed: 11738635]
62. Henning SJ. Ontogeny of enzymes in the small intestine. Annu Rev Physiol. 1985; 47:231–245. [PubMed: 3888075]
63. Falk P, Roth KA, Gordon JI. Lectins are sensitive tools for defining the differentiation programs of mouse gut epithelial cell lineages. Am J Physiol. 1994; 266:G987–G1003. [PubMed: 8023947]
64. Cheesman SE, Neal JT, Mittge E, et al. Epithelial cell proliferation in the developing zebrafish intestine is regulated by the Wnt pathway and microbial signaling via Myd88. Proc Natl Acad Sci U S A. 2011; 108(suppl 1):4570–4577. [PubMed: 20921418]
65. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 2004; 118:229–241. [PubMed: 15260992]
66. Gibson DL, Ma C, Bergstrom KS, et al. MyD88 signalling plays a critical role in host defence by controlling pathogen burden and promoting epithelial cell homeostasis during Citrobacter rodentium-induced colitis. Cell Microbiol. 2008; 10:618–631. [PubMed: 17979981]
67. Pull SL, Doherty JM, Mills JC, et al. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. Proc Natl Acad Sci U S A. 2005; 102:99–104. [PubMed: 15615857]
68. Hikasa H, Sokol SY. Wnt signaling in vertebrate axis specification. Cold Spring Harb Perspect Biol. 2013; 5:a007955. [PubMed: 22914799]
69. Carten JD, Bradford MK, Farber SA. Visualizing digestive organ morphology and function using differential fatty acid metabolism in live zebrafish. Dev Biol. 2011; 360:276–285. [PubMed: 21968100]
70. Walters JW, Anderson JL, Bittman R, et al. Visualization of lipid metabolism in the zebrafish intestine reveals a relationship between NPC1L1-mediated cholesterol uptake and dietary fatty acid. Chem Biol. 2012; 19:913–925. [PubMed: 22749558]
71. Galindo-Villegas J, Garcia-Moreno D, de Oliveira S, et al. Regulation of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development. Proc Natl Acad Sci U S A. 2012; 109:E2605–E2614. [PubMed: 22949679]

72. Bates JM, Akerlund J, Mittge E, et al. Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. Cell Host Microbe. 2007; 2:371–382. [PubMed: 18078689]
73. Yang Y, Wandler AM, Postlethwait JH, et al. Dynamic evolution of the LPS-detoxifying Enzyme intestinal alkaline phosphatase in zebrafish and other vertebrates. Front Immunol. 2012; 3:314. [PubMed: 23091474]
74. Carnevali O, Avella MA, Gioacchini G. Effects of probiotic administration on zebrafish development and reproduction. Gen Comp Endocrinol. 2013; 188:297–302. [PubMed: 23500006]
75. Rendueles O, Ferrieres L, Fretaud M, et al. A new zebrafish model of oro-intestinal pathogen colonization reveals a key role for adhesion in protection by probiotic bacteria. PLoS Pathog. 2012; 8:e1002815. [PubMed: 22911651]
76. Brugman S, Liu KY, Lindenbergh-Kortleve D, et al. Oxazolone-induced enterocolitis in zebrafish depends on the composition of the intestinal microbiota. Gastroenterology. 2009; 137:1757–1767. e1751. [PubMed: 19698716]
77. Hoentjen F, Harmsen HJ, Braat H, et al. Antibiotics with a selective aerobic or anaerobic spectrum have different therapeutic activities in various regions of the colon in interleukin 10 gene deficient mice. Gut. 2003; 52:1721–1727. [PubMed: 14633949]
78. Kanther M, Tomkovich S, Sun X, et al. Commensal microbiota stimulate systemic neutrophil migration through induction of Serum amyloid A. Cell Microbiol. published online ahead of print December 26, 2013. 10.1111/cmi.12257
79. Heller F, Fuss IJ, Nieuwenhuis EE, et al. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. Immunity. 2002; 17:629–638. [PubMed: 12433369]
80. Boirivant M, Fuss IJ, Chu A, et al. Oxazolone colitis: a murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. J Exp Med. 1998; 188:1929–1939. [PubMed: 9815270]
81. Packey CD, Sartor RB. Interplay of commensal and pathogenic bacteria, genetic mutations, and immunoregulatory defects in the pathogenesis of inflammatory bowel diseases. J Intern Med. 2008; 263:597–606. [PubMed: 18479259]
82. Crawford KC, Vega Flores M, Oehlers SH, et al. Zebrafish heat shock protein a4 genes in the intestinal epithelium are up-regulated during inflammation. Genesis. 2011; 49:905–911. [PubMed: 21557452]
83. Fleming A, Jankowski J, Goldsmith P. In vivo analysis of gut function and disease changes in a zebrafish larvae model of inflammatory bowel disease: a feasibility study. Inflamm Bowel Dis. 2010; 16:1162–1172. [PubMed: 20128011]
84. Oehlers SH, Flores MV, Hall CJ, et al. Expression of zebrafish cxcl8 (interleukin-8) and its receptors during development and in response to immune stimulation. Dev Comp Immunol. 2010; 34:352–359. [PubMed: 19941893]
85. Oehlers SH, Flores MV, Okuda KS, et al. A chemical enterocolitis model in zebrafish larvae that is dependent on microbiota and responsive to pharmacological agents. Dev Dyn. 2011; 240:288–298. [PubMed: 21181946]
86. Oehlers SH, Flores MV, Hall CJ, et al. Retinoic acid suppresses intestinal mucus production and exacerbates experimental enterocolitis. Dis Model Mech. 2012; 5:457–467. [PubMed: 22563081]
87. Araki A, Kanai T, Ishikura T, et al. MyD88-deficient mice develop severe intestinal inflammation in dextran sodium sulfate colitis. J Gastroenterol. 2005; 40:16–23. [PubMed: 15692785]
88. Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, et al. Amelioration of chronic ileitis by nitric oxide synthase inhibition. J Pharmacol Exp Ther. 1993; 264:11–16. [PubMed: 7678645]
89. Pilichos CJ, Kouerinis IA, Zografos GC, et al. The effect of nitric oxide synthases inhibitors on inflammatory bowel disease in a rat model. In Vivo. 2004; 18:513–516. [PubMed: 15369194]
90. Zlotnik A, Yoshie O, Nomiyama H. The chemokine and chemokine receptor superfamilies and their molecular evolution. Genome Biol. 2006; 7:243. [PubMed: 17201934]
91. Mizushima T. HSP-dependent protection against gastrointestinal diseases. Curr Pharm Des. 2010; 16:1190–1196. [PubMed: 20166990]

92. Nakamura K, Rokutan K, Marui N, et al. Induction of heat shock proteins and their implication in protection against ethanol-induced damage in cultured guinea pig gastric mucosal cells. Gastroenterology. 1991; 101:161–166. [PubMed: 2044905]

93. Otaka M, Odashima M, Tamaki K, et al. Expression and function of stress (heat shock) proteins in gastrointestinal tract. Int J Hyperthermia. 2009; 25:634–640. [PubMed: 20021224]

94. Oyake J, Otaka M, Matsuhashi T, et al. Over-expression of 70-kDa heat shock protein confers protection against monochloramine-induced gastric mucosal cell injury. Life Sci. 2006; 79:300–305. [PubMed: 16492383]

95. Faure M, Moennoz D, Montigon F, et al. Mucin production and composition is altered in dextran sulfate sodium-induced colitis in rats. Dig Dis Sci. 2003; 48:1366–1373. [PubMed: 12870797]

96. Perse M, Cerar A. Dextran sodium sulphate colitis mouse model: traps and tricks. J Biomed Biotechnol. 2012; 2012:718617. [PubMed: 22665990]

97. Maiden L, Thjodleifsson B, Theodors A, et al. A quantitative analysis of NSAID-induced small bowel pathology by capsule enteroscopy. Gastroenterology. 2005; 128:1172–1178. [PubMed: 15887101]

98. Morteau O, Morham SG, Sellon R, et al. Impaired mucosal defense to acute colonic injury in mice lacking cyclooxygenase-1 or cyclooxygenase-2. J Clin Invest. 2000; 105:469–478. [PubMed: 10683376]

99. Goldsmith JR, Cocchiaro JL, Rawls JF, et al. Glafenine-induced intestinal injury in zebrafish is ameliorated by mu-opioid signaling via enhancement of Atf6-dependent cellular stress responses. Dis Model Mech. 2013; 6:146–159. [PubMed: 22917923]

100. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nat Rev Immunol. 2013; 13:722–737. [PubMed: 24064518]

101. Goldsmith JR, Uronis JM, Jobin C. Mu opioid signaling protects against acute murine intestinal injury in a manner involving Stat3 signaling. Am J Pathol. 2011; 179:673–683. [PubMed: 21801866]

102. Stockhammer OW, Zakrzewska A, Hegedus Z, et al. Transcriptome profiling and functional analyses of the zebrafish embryonic innate immune response to Salmonella infection. J Immunol. 2009; 182:5641–5653. [PubMed: 19380811]

103. van der Sar AM, Stockhammer OW, van der Laan C, et al. MyD88 innate immune function in a zebrafish embryo infection model. Infect Immun. 2006; 74:2436–2441. [PubMed: 16552074]

104. Feng CG, Scanga CA, Collazo-Custodio CM, et al. Mice lacking myeloid differentiation factor 88 display profound defects in host resistance and immune responses to Mycobacterium avium infection not exhibited by Toll-like receptor 2 (TLR2)- and TLR4-deficient animals. J Immunol. 2003; 171:4758–4764. [PubMed: 14568952]

105. Issac JM, Sarawathiamma D, Al-Ketbi MI, et al. Differential outcome of infection with attenuated Salmonella in MyD88-deficient mice is dependent on the route of administration. Immunobiology. 2013; 218:52–63. [PubMed: 22386951]

106. Ko HJ, Yang JY, Shim DH, et al. Innate immunity mediated by MyD88 signal is not essential for induction of lipopolysaccharide-specific B cell responses but is indispensable for protection against Salmonella enterica serovar Typhimurium infection. J Immunol. 2009; 182:2305–2312. [PubMed: 19201885]

107. Park SM, Ko HJ, Shim DH, et al. MyD88 signaling is not essential for induction of antigen-specific B cell responses but is indispensable for protection against Streptococcus pneumoniae infection following oral vaccination with attenuated Salmonella expressing PspA antigen. J Immunol. 2008; 181:6447–6455. [PubMed: 18941235]

108. Scanga CA, Bafica A, Feng CG, et al. MyD88-deficient mice display a profound loss in resistance to Mycobacterium tuberculosis associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. Infect Immun. 2004; 72:2400–2404. [PubMed: 15039368]

109. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucinerich repeat variants with susceptibility to Crohn's disease. Nature. 2001; 411:599–603. [PubMed: 11385576]

110. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature. 2001; 411:603–606. [PubMed: 11385577]

111. Hasegawa M, Yamazaki T, Kamada N, et al. Nucleotide-binding oligomerization domain 1 mediates recognition of Clostridium difficile and induces neutrophil recruitment and protection against the pathogen. J Immunol. 2011; 186:4872–4880. [PubMed: 21411735]
112. Kim YG, Shaw MH, Warner N, et al. Cutting edge: Crohn's disease-associated Nod2 mutation limits production of proinflammatory cytokines to protect the host from Enterococcus faecalis-induced lethality. J Immunol. 2011; 187:2849–2852. [PubMed: 21849681]
113. Mantovani A, Cassatella MA, Costantini C, et al. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol. 2011; 11:519–531. [PubMed: 21785456]
114. Ribeiro CM, Hermsen T, Taverne-Thiele AJ, et al. Evolution of recognition of ligands from Gram-positive bacteria: similarities and differences in the TLR2-mediated response between mammalian vertebrates and teleost fish. J Immunol. 2010; 184:2355–2368. [PubMed: 20118281]
115. Thakur PC, Davison JM, Stuckenholz C, et al. Dysregulated phosphatidylinositol signaling promotes endoplasmic reticulum stress-mediated intestinal mucosal injury and inflammation in zebrafish. Dis Model Mech. 2013; 86:1133–1149.
116. Adolph TE, Tomczak MF, Niederreiter L, et al. Paneth cells as a site of origin for intestinal inflammation. Nature. 2013; 503:272–276. [PubMed: 24089213]
117. Kaser A, Lee AH, Franke A, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell. 2008; 134:743–756. [PubMed: 18775308]
118. Cahill CM, Rogers JT, Walker WA. The role of phosphoinositide 3-kinase signaling in intestinal inflammation. J Signal Transduct. 2012; 2012:358476. [PubMed: 22570785]
119. Prescott D, Atkinson B, Doring A, et al. Loss of phosphoinositide 3-kinase P110gamma is protective in the acute phase but detrimental in the resolution phase of hapten-induced colitis. Inflamm Bowel Dis. 2013; 19:489–500. [PubMed: 23282580]
120. Uno JK, Rao KN, Matsuoka K, et al. Altered macrophage function contributes to colitis in mice defective in the phosphoinositide-3 kinase subunit p110delta. Gastroenterology. 2010; 139:1642–1653. 1653 e1641–e1646. [PubMed: 20637203]
121. Niu X, Gao C, Jan Lo L, et al. Sec13 safeguards the integrity of the endoplasmic reticulum and organogenesis of the digestive system in zebrafish. Dev Biol. 2012; 367:197–207. [PubMed: 22609279]
122. Fleming A, Rubinsztein DC. Zebrafish as a model to understand autophagy and its role in neurological disease. Biochim Biophys Acta. 2011; 1812:520–526. [PubMed: 21256213]
123. Holt A, Mitra S, van der Sar AM, et al. Discovery of zebrafish (Danio rerio) interleukin-23 alpha (IL-23alpha) chain, a subunit important for the formation of IL-23, a cytokine involved in the development of Th17 cells and inflammation. Mol Immunol. 2011; 48:981–991. [PubMed: 21324528]
124. Hundorfean G, Neurath MF, Mudter J. Functional relevance of T helper 17 (Th17) cells and the IL-17 cytokine family in inflammatory bowel disease. Inflamm Bowel Dis. 2012; 18:180–186. [PubMed: 21381156]
125. Kuhn R, Lohler J, Rennick D, et al. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993; 75:263–274. [PubMed: 8402911]
126. Glocker EO, Kotlarz D, Boztug K, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med. 2009; 361:2033–2045. [PubMed: 19890111]
127. Moran CJ, Walters TD, Guo CH, et al. IL-10R polymorphisms are associated with very-early-onset ulcerative colitis. Inflamm Bowel Dis. 2013; 19:115–123. [PubMed: 22550014]
128. Grayfer L, Belosevic M. Identification and molecular characterization of the interleukin-10 receptor 1 of the zebrafish (Danio rerio) and the goldfish (Carassius auratus L.). Dev Comp Immunol. 2012; 36:408–417. [PubMed: 21906622]
129. Zhang DC, Shao YQ, Huang YQ, et al. Cloning, characterization and expression analysis of interleukin-10 from the zebrafish (Danio rerion). J Biochem Mol Biol. 2005; 38:571–576. [PubMed: 16202237]
130. Kettleborough RN, Busch-Nentwich EM, Harvey SA, et al. A systematic genome-wide analysis of zebrafish protein-coding gene function. Nature. 2013; 496:494–497. [PubMed: 23594742]

131. Brand S, Beigel F, Olszak T, et al. IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. Am J Physiol Gastrointest Liver Physiol. 2006; 290:G827–G838. [PubMed: 16537974]

132. Mizoguchi A. Healing of intestinal inflammation by IL-22. Inflamm Bowel Dis. 2012; 18:1777–1784. [PubMed: 22359410]

133. Costa MM, Saraceni PR, Forn-Cuni G, et al. IL-22 is a key player in the regulation of inflammation in fish and involves innate immune cells and PI3K signaling. Dev Comp Immunol. 2013; 41:746–755. [PubMed: 23999050]

134. Toh MC, Goodyear M, Daigneault M, et al. Colonizing the embryonic zebrafish gut with anaerobic bacteria derived from the human gastrointestinal tract. Zebrafish. 2013; 10:194–198. [PubMed: 23530761]

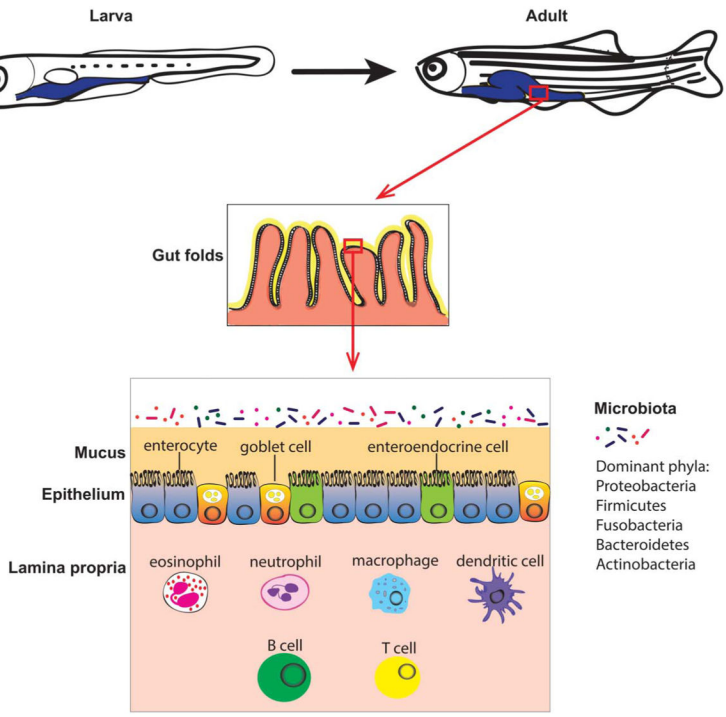

FIGURE 1.

The zebrafish intestine and gut microbiota. The zebrafish intestine (outlined in blue) undergoes rapid development, from a linear tube in the larva to a complex organ that folds twice in the abdominal cavity in the adult. The intestinal epithelium forms irregular ridge structures (gut folds) and is composed of 3 differentiated cell types, that is, the absorptive enterocytes, mucus-producing goblet cells, and secretory enteroendocrine cells. Underneath the epithelium is the lamina propria, which harbors various immune cells implicated in host–microbe interactions in the gut. The zebrafish has a complex gut microbiota, with the bacterial phyla Proteobacteria, Firmicutes, Fusobacteria, Bacteroidetes, and Actinobacteria being dominantly present in laboratory-reared fish among different research facilities.

TABLE 1

Zebrafish Models of Intestinal Immune Disorders

	Age	Phenotype	References
Chemically Induced Models			
Oxazolone	Adult	Intestinal structure disruption	76
		Goblet cell (GC) depletion	
		Neutrophil and eosinophil recruitment to intestine	
		Upregulated proinflammatory genes (*il-1β*, *tnf-α*, *il-10*)	
TNBS	3–5 dpf	Impaired gut function	82–86
		Increased GC proliferation	
		Leukocyte enrichment and recruitment to intestine	
		Upregulated proinflammatory genes (*il-1β*, *tnf-α*, *mmp9*)	
		Increased NO production	
DSS	3–6 dpf	Increased GC proliferation	86
		Neutrophil recruitment to intestine	
		Mucus accumulation	
		Upregulated proinflammatory genes (*il-1β*, *tnf-α*, *mmp9*, *il*-8, *ccl20*)	
Glafenine	5 dpf	Intestinal epithelial cell apoptosis	99
		ER stress in intestinal epithelial cells	
Genetic models			
myd88-MO knockdown and mutant	2–6 dpf	Increased susceptibility to bacterial infections	51,102,103
		Impaired proinflammatory gene expression (*il-1β* and *mmp9)*	
nod1/2 MO knockdown	2–5 dpf	Increased susceptibility to bacterial infections	50
		Impaired dual oxidase expression	
cdipt^{hi559} mutant	3–6 dpf	Intestinal structure disruption	115
		Apoptotic GCs	
		Inflammation	
		Bacterial overgrowth	
		ER stress in intestinal epithelial cells	
sec13^{sq198} mutant	2–5 dpf	Impaired intestine development	121
		ER stress in intestinal epithelial cells	
il-23 expression	Adult	Upregulated in response to LPS or bacterial infection	123
il-10 expression	Adult	Upregulated in response to LPS	128,129
il-22-MO knockdown	3–6 dpf	Increased susceptibility to bacterial infection	133
		Increased proinflammatory gene expression (*il-1β* and *tnf-α*)	