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Utilization of zebrafish for intravital study of eukaryotic pathogen–host interactions

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Abstract

Unique imaging tools and practical advantages have made zebrafish a popular model to investigate *in vivo* host–pathogen interactions. These studies have uncovered details of the mechanisms involved in several human infections. Until recently, studies using this versatile host were limited to viral and prokaryotic pathogens. Eukaryotic pathogens are a diverse group with a major impact on the human and fish populations. The relationships of eukaryote pathogens with their hosts are complex and many aspects remain obscure. The small and transparent zebrafish, with its conserved immune system and amenability to genetic manipulation, make it an exciting model for quantitative study of the core strategies of eukaryotic pathogens and their hosts. The only thing to do now is realize its potential for advancement of biomedical and aquaculture research.

Keywords

Zebrafish; Immunity; Fungal; Protozoan; Helminth; Host–pathogen; Candida

1. Introduction

Eukaryotic microbes and helminths are among the most important human pathogens worldwide. Fungi account for hundreds of thousands of deaths each year and significant clinical burden in developed economies (Brown et al., 2012; Enoch et al., 2006). Malaria affects millions each year and its historical significance has made a tremendous footprint on the modern human genome (Sabeti et al., 2002). Other protozoan pathogens infect hundreds of millions and their burden reaches far beyond mortality statistics (Lupi et al., 2009). *Trypanosoma brucei* has caused four major epidemics and the World Health Organization estimates that 60 million humans are at risk in central Africa (Simarro et al., 2008). In the developed world, serious protozoan infection outbreaks have been traced to *Giardia duodenalis* and *Cryptosporidium parvum*, which cause waterborne infections (Karnis et al., 2007). Parasitic worms also chronically infect hundreds of millions and are the most

common infectious agents in the developing countries (Hotez et al., 2008). But not all parasites are pathogens, and based on their known effects on the immune homeostasis it is believed that we have evolved to accommodate chronic helminths infection so that in its absence we are at a higher risk for autoimmune disease (Jackson et al., 2009).

The zebrafish larva has become a powerful model host to understand host–pathogen interaction in the vertebrate. Tremendous progress has been made with bacterial and mycobacterial infections, and recent work suggests that similar strides can be made in discovery of the mechanisms underlying the pathogenesis of disease caused by eukaryotic pathogens. Despite the fact that eukaryotic pathogens make such a large footprint on human populations worldwide, we still have limited mechanistic understanding of how they dynamically interact with their host during infection. In this review, we make the case that the larval zebrafish model represents a unique opportunity to transform our knowledge of some key aspects of eukaryotic virulence and host immunity to eukaryotic pathogens.

2. An appraisal of zebrafish as a model host for eukaryotic infection

The zebrafish larva is a model system with unique advantages but important limitations. Exploitation of the unique aspects of the system have led to important advances in our understanding of human diseases, including infectious disease.

Practically, zebrafish are an enviable experimental animal because they are small, cheap, and relatively easy to raise. Just as mice before them, they have been and remain a popular pet because of their hardiness. It is only model organism for which you can purchase a green or red glowing transgenic animal at your local pet store for just a few dollars. This ease of husbandry, combined with the ability to fit larvae in a 96-well plate, lends itself to high-throughput chemical and genetic screening, two techniques that have been applied to great effect already in zebrafish models of infectious disease (Clatworthy et al., 2007; d'Alencon et al., 2010; Mullins et al., 1994; Takaki et al., 2012; Tan and Zon, 2011; Tobin et al., 2010).

On the technical side, there are a large number of fairly standard techniques that can be applied to these studies, although there are some limitations in zebrafish. Genetic work is highly advanced, but not quite to the same level as the mouse. The zebrafish genome has been well sequenced, assembled and annotated, although there are still a large number of gaps. Genome engineering is now feasible with zinc-finger nucleases, TALENs, and Cas-CRISPR modifications, but there are as yet no engineered knock-out or knock-in lines that form the backbone for mouse research (Blackburn et al., 2013; Campbell et al., 2013). In addition, there are few tissue-specific promoters or lines for driving tissue-specific expression. In recompense, it is possible to use morpholino oligonucleotides to alter gene splicing and translation with ease, but this technology is relatively expensive, limited to the first week of development, and prone to artifacts.

Where this model really shines is in the regime of intravital microscopy, where it is unparalleled. Deep tissue in the transparent larva can be imaged on a time frame of milliseconds to days in four-dimensions with light sheet, total internal reflection, spinning disk, two-photon, or laser-scanning confocal microscopy. This results in sub-cellular resolution of fluorescence signal throughout the host and pathogen, revealing host–pathogen

dynamics at a scale that is not possible in any other vertebrate system. Limitations of the mouse model include the need to externalize tissue or use culture tissue slices to image organs, limitation on the length of anesthesia, and reduced resolution for deeper imaging. Application of improved imaging techniques will eventually allow deeper imaging at higher resolution in the mouse, and correspondingly enhanced imaging prowess in the zebrafish.

These transformational imaging approaches in zebrafish enable new categories of questions to be asked about infection *in vivo*, in real time. These include questions of disease architecture, microenvironmental constraints in different tissues (e.g. hypoxia, glucose starvation, alternate carbon source utilization, local immunosuppression), and the roles of host and pathogen morphogenesis and movement in disease. The successful exploitation of the zebrafish model to reveal new aspects of human infectious diseases and disorders also speaks for itself (Ablain and Zon, 2013; Cui et al., 2011; Santoriello and Zon, 2012; Sullivan and Kim, 2008).

3. Current zebrafish models for human infections with pathogenic eukaryotes

3.1. Fungal pathogens

The high global burden of eukaryotic infectious diseases for human health has led to a new focus on understanding both the pathogens themselves and the diseases they cause, with the intention of curing these worldwide problems. To date, experimental work with eukaryotic pathogens in zebrafish has focused on fungi, a very important group of eukaryotic disease organisms in the developed world and of high importance globally. Fungi cause fatal disease throughout the plant and animal kingdoms, and several pose challenging problems in the United States and around the world (Brown et al., 2012). For the most part, fungal disease in humans results from either high-dose inoculums or impaired immunity. The HIV/AIDS epidemic and cancer treatments have created a large immunocompromised population that frequently succumbs to fungal opportunistic pathogens. Cryptococcosis fells hundreds of thousands of AIDS patients per year, and oral candidiasis and pneumocystis pneumonia (PCP) affect many of these same patients. In the United States and Europe, the fungal pathogen with the greatest clinical impact is *Candida albicans*. It is a ubiquitous human gut commensal that causes frequent invasive disease in patients immunocompromised by transplants, chemotherapy, and in-dwelling medical devices (Enoch et al., 2006).

Experimental infection models of both adult and larval zebrafish with *C. albicans* have been developed (Brothers et al., 2011; Chao et al., 2010). Initial experiments have also been undertaken to model virulence of the human fungal pathogens *Penicillium marneffei*, *Aspergillus fumigatus* and *Cryptococcus neoformans* (Berthier et al., 2013; Ellett et al., 2011; Johnston and May, Personal communication). The larval candidiasis model has proven useful to dissect host and pathogen disease determinants, and the adult model of candidemia has yielded genome-wide expression patterns in *C. albicans* and whole zebrafish during infection.

Two different larval models have been developed, one to mimic disseminated candidiasis upon hindbrain infection and one to model mucosal candidiasis upon swimbladder infection.

Work in the disseminated model uncovered two unexpected aspects of host response to fungal infection. First, it was found that macrophages can limit fungal germination of engulfed yeast (Brothers et al., 2011). This is something that is not found when macrophages are challenged with *C. albicans* *in vitro*, with either mammalian or zebrafish macrophages ((Lorenz et al., 2004), Hopke and Wheeler unpublished data). This suggests that macrophages may play a more active role than previously envisioned in host defense against candidemia (Lionakis and Netea, 2013; Lionakis et al., 2013). Second, a new role for NADPH oxidase activity in recruitment of immune cells to the site of infection was described (Brothers et al., 2013). Remarkably, morpholino knockdown of either epithelial *Duox* or phagocyte *Phox* resulted in reduced early phagocyte recruitment to fungal infection. The consequences of this lost recruitment and containment were lethal to the host, and included germination of extracellular fungi and extensive tissue invasion by hyphal filaments. These initial mechanistic studies highlight unexpectedly important aspects of early *in vivo* *Candida*-phagocyte interaction that remain inaccessible in the mouse models of candidemia.

The zebrafish swimbladder model of candidiasis opens up a new methodology of experimental infection for dissection of host and pathogen determinants at the epithelial barrier. This model of mucosal candidiasis recapitulates key aspects of *C. albicans*-epithelial interaction known from *in vitro* and *in vivo* experiments with mammalian cells, including activation of NF- κ B signaling, inflammatory cytokine expression and neutrophil recruitment (Gratacap et al., 2013). Recently, a microinjection model of swimbladder candidiasis has also been described, which expands the range of questions that can be asked in this tissue (Brothers et al., 2013). This mucosal infection model adds a new tool to the zebrafish larval infection arsenal, and can be easily adapted to use intravital imaging in the study of other pathogens that infect mucosal surfaces, including the vast majority of environmental infections. Study of infection in this tissue compartment may reveal mechanisms underlying how pathogens interact with and pass through the mucosal barrier to disseminate throughout the host.

The adult infection model has been used to characterize gene expression patterns in both the host and *C. albicans*. It was shown that *C. albicans* induces genes associated with filamentous growth as it invades tissue with hyphae following intraperitoneal infection. Then it was shown that key regulators of the filamentous growth regulatory pathway are required for invasion, as was also demonstrated for the larval hindbrain model (Brothers et al., 2011; Chao et al., 2010). More recent work has used genome-wide expression data to identify potential networks of genes that regulate either *C. albicans* virulence or host immune response (Chen et al., 2013; Kuo et al., 2013; Wang et al., 2013). These studies have used sophisticated bioinformatics tools to overlay gene expression changes with protein-protein interaction matrices and thereby make unique predictions as to networks and processes that may be important during infection.

3.2. Other pathogenic eukaryotes

Protozoan pathogens have a limited impact in the developed world but have had a much greater, more sustained footprint worldwide. Protozoan pathogenesis has been studied in

organisms from *Drosophila* to humans, yet very little has been done with zebrafish to date. The only published experimental infections of zebrafish with human protozoan pathogens have been gavages of adult fish with *G. duodenalis*. Here it was shown that *G. duodenalis* can colonize the zebrafish gut for several days but does not seem to cause robust infection (Tysnes et al., 2012). Although ciliates are a natural food source for zebrafish larvae, recent work hints that they can also be vectors for infection (Peterson et al., 2013). Here it was found that *Mycobacterium*-infected *Paramecium caudatum* can cause disease upon addition to water, whereas mycobacteria alone added to the water did not result in disease. These initial studies suggest that studying protozoans in the zebrafish may reveal novel aspects of microbe–microbe interactions in disease. Study of both human pathogens and natural fish pathogens in zebrafish may prove fruitful.

4. Natural eukaryotic and metazoan infections of zebrafish and other fish

4.1. Relevance to aquaculture

Fish infection is important in the wild, in marine aquaculture and land-based fish farms. Wild fish are being caught to extinction, and more fish need to be farmed instead of caught. Farmed fish are susceptible to infectious diseases, caused by viral, bacterial, protozoan and metazoan pathogens. High-density farming makes epidemics rapid and catastrophic, while chronic diseases are also a concern. The largest production of a single species in aquaculture is the carp, and parasitic infections with the blood fluke have been shown to decrease the food conversion ratio and significantly impact production (Iqbal and Sommerville, 1986). The most efficient way to avoid disease in aquaculture is through vaccination. The zebrafish's small size, short generation time and phylogenetic proximity to the carp make it a good model for vaccine testing. Several studies have used zebrafish to test viral and bacterial vaccines (Novoa et al., 2006; Zhang et al., 2012). However, to date, no studies have investigated the use of zebrafish as a model for vaccine design and testing against eukaryotic infections. A greater understanding of fish infection and immunity in the realm of eukaryotic pathogens has the potential to propel this industry forward in its bid to replace overtaxed wild fisheries.

4.2. Natural eukaryotic pathogens of zebrafish

The use of zebrafish as both pets and laboratory animals has led to excellent studies of natural pathogens in aquarium settings. Work led by the aquaculture pathologists in Oregon has identified fungal, parasitic and metazoan infections of adult zebrafish (Kent et al., 2002; Matthews, 2004; Matthews et al., 2001 Table 1). One infectious pathogen class of significant interest has been the microsporidians, as there are relatives that infect worms and humans. These obligate intracellular fungi are closely related to zygomycetes and have an unusual mechanism of infection, with a polar, coiled, tube firing into the target cell and directly injecting the pathogen (Lee et al., 2008; Troemel, 2011). Microsporidia have a “bare bones” genome, and are therefore an interesting model of genome reduction (Troemel, 2011). Experimental infections in zebrafish with *Pseudoloma neurophilia* have revealed its ability to infect nervous and muscle tissue, and led to a detailed description of its development in the infected host (Cali et al., 2012). Recent work has also characterized a vertical mode of transmission for this fungus (Sanders et al., 2013). Study of

microsporidians may have implications for host–pathogen evolution, human disease, and gut-microbe interactions. Another infection of more general significance is that of the nematode *Pseudocapillaria tomentosa*, which infects research colonies. Remarkably, *P. tomentosa* infection is associated with cancer initiation and progression (Kent et al., 2002). This is particularly interesting because of the recently established links between microbe-associated inflammation and gut cancers in humans (Arthur et al., 2012; Stein et al., 2013; Zackular et al., 2013). *P. tomentosa* has also been experimentally used to study Th2 immunity and more particularly the characterization of zebrafish eosinophils (Balla et al., 2010). Zebrafish models of these aquarium-associated infections have the opportunity to shed light on immunity to fungi and helminths, the role of helminths in immune balance, and both human and fish health.

Zebrafish also suffer from a number of parasites in the wild, although this has not been extensively studied. Fish as a class are known to be heavily infected by nematodes, as described for the three spine stickleback (Barber, 2013), and the large number of natural helminth parasites of zebrafish highlight the potential to exploit this to study the relationship between immunity and nematode. A large survey in the Ganges River in India identified metazoan parasites from 23 taxa infecting wild zebrafish (Smith et al., 2011). These were chiefly larval digeneans, including metacercariae from the genera *Acanthostomum* and *Centrocestus*. While their study focused on metazoan parasites, these authors also noted ciliate and myxozoan infections by *Myxobolus* and *Thelohanellus*. Although no protozoan infections of zebrafish have been described to date, the difficulties associated with diagnosis in challenging field conditions of the natural range of zebrafish (the Indo-Bangladeshi subcontinent) leave this possibility open.

4.3. Eukaryotic pathogens of commercially valuable cyprinid fish species

Given our lack of knowledge about natural eukaryotic pathogens of zebrafish, another way to discover zebrafish pathogens suitable for modeling human disease could be to use pathogens of fish species closely related to the zebrafish (Woo and Bachmann, 2012). Related cyprinid species include the common carp (*Cyprinus carpio*), the goldfish (*Carassius auratus*), and fish more adapted to tropical environments, such as Indian carp (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*). One such study in silver carp identified infections with *Gyrodactylus* and *Dactylogyrus*, both monogeneans, as well as the ciliate *Trichodina* in wild fish (Alam et al., 2012). Of particular interest from the human health perspective, common carp and goldfish are natural hosts for *Trypanosoma carassii* and *T. borreli* (Overath et al., 1998; Ribeiro et al., 2010). It may be possible to use natural pathogens of these closely related fish species to model disease in the zebrafish and thereby get a different perspective on related human pathogens.

5. Immune responses to eukaryotic infections

The vertebrate immune system is well conserved from fish to humans, especially in terms of cell types, development, signaling pathways and intercellular signals (Bennett et al., 2001; Lieschke, 2001; Lieschke et al., 2001; Stachura and Traver, 2011; Stein et al., 2007; Sullivan and Kim, 2008). This conservation is schematically shown in Fig. 1. Zebrafish have Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), although the direct

correspondence with mammalian TLRs or CLRs has not yet been established (Roach et al., 2005; Vasta et al., 2011; Zelensky and Gready, 2005). Preliminary studies in our laboratory suggest that there is at least one CLR that recognizes the fungus *C. albicans* (EV Carter and RTW, unpublished work), and based on the multipronged recognition of fungi in mammals (Netea et al., 2008) we expect to identify more such receptors in future functional work.

The roles of cytokines associated with T-helper differentiation are well conserved between zebrafish and humans, although their roles in fish infection have not yet been functionally established (Scapigliati, 2013; Wang and Secombes, 2013). The potential for these cytokines to play roles in both innate and adaptive immunity suggest that studies in both the larval and adult zebrafish will be useful. Of particular importance for studies of eukaryotic pathogens, the IL-17 and IL-22 pathways are present in zebrafish, and mast cells, dendritic cells and eosinophils have all been described (Balla et al., 2010; Dobson et al., 2008; Gunimaladevi et al., 2006; Igawa et al., 2006; Wittamer et al., 2011). Intriguingly, IL-17 signaling, of high importance in anti-fungal immunity in humans, has been implicated in response to infection in zebrafish (Hernandez-Santos and Gaffen, 2012; Puel et al., 2012; Zhang et al., 2013). Consistent with a potential innate role for this pathway, recent work suggests that both IL-17 and IL-22 are expressed in juvenile zebrafish, at a development stage where the adaptive immune system is not fully functional (Costa et al., 2013; Danilova et al., 2004; Lam et al., 2004; Page et al., 2013; Quintana et al., 2010). Work in carp has implicated IL-17 and IL-23 in response to trypanosomes, along with the expected recruitment of neutrophils (Ribeiro et al., 2010). It remains to be seen if these signaling pathways also play key roles in immunity to eukaryotic infectious agents in the zebrafish.

It has long been known that the Th2 response pathway is activated by helminths and several parasites, with a consequent downregulation of Th1 responses (Pearce et al., 1991). Nevertheless, relatively little is known in the zebrafish regarding the Th2 pathway and eosinophil activity during infection. Conservation of eosinophil function is suggested by recent experiments in zebrafish. Their eosinophils degranulate and release peroxidase after activation with helminth extract and infection with the helminth *Pseudocapillaria tomentosa* induces eosinophilia to the intestine (Balla et al., 2010; Zhu et al., 2013). Reinforcing the contention that vertebrate T helper cell differentiation is conserved, it was recently shown that zebrafish express *stat6* (along with *t-bet* and *foxp3*), (Mitra et al., 2010). IL-4 is a prototypical cytokine required for Th2 type response and injection of the recombinant protein into live zebrafish induces Th2 type cytokines and regulators (*il10*, *stat6*, *gata3* and *c-maf*) and downregulates Th1 type cytokines and regulators (*irf1*, *t-bet*, *stat1* and *ifng*) (Zhu et al., 2012).

More is known about commercially valuable fish species and their immune responses to natural fish parasites (Alvarez-Pellitero, 2008). Translation of this knowledge may allow for future work to utilize the practical advantages of the zebrafish. The juvenile zebrafish holds promise in study of the relationships of eukaryotic pathogens with hematopoiesis and the innate immune system. In addition, transparent adult models such as the Casper strain, in conjunction with new genome engineering techniques, will empower future studies probing adaptive immunity to human and fish pathogens (White et al., 2008).

6. Looking forward: how could zebrafish studies further illuminate human infectious disease?

6.1. Use of natural eukaryotic zebrafish pathogens to understand closely related human pathogens

Using a natural fish pathogen has significant advantages over using a human pathogen when the human pathogen is significantly host-restricted. First, the pathogen has successfully evolved mechanisms to infect the zebrafish. Since some of these mechanisms are likely to be at least somewhat host-specific, one can identify virulence mechanisms and use translational models to characterize general mechanisms of virulence for that class of pathogen. Second, the pathogen has adapted to the appropriate temperature and environmental parameters of the host. If virulence factors are regulated by temperature, these pathogens will have the ability to regulate them properly at the ideal temperature for zebrafish husbandry, 28 °C. Exploitation of these advantages using the fish pathogen *Mycobacterium marinum* to model human mycobacterial diseases has revealed core aspects of mycobacterial disease with clear and rapid translational value (Tobin et al., 2012b, 2010). However, few natural pathogens of the zebrafish are well understood or tractable, so for the study of particular infectious agents it is necessary to study infection of zebrafish with the human pathogen.

There are several eukaryotic fish pathogens with close relatives that cause devastating disease in human. Possibly the closest-related human pathogens infecting fish are from the *Trypanosoma* family. In humans, *T. brucei* causes sleeping sickness and *T. cruzi* causes Chagas disease. Both *T. carassii* and *T. borreli* infect goldfish and carp, where they are found in the blood and are believed to be extracellular pathogens (Overath et al., 1998; Ribeiro et al., 2010). Interestingly, it seems that *T. carassii* induces a Th17-type response (Ribeiro et al., 2010) whereas *T. borreli* activates a Th1-type response (Forlenza et al., 2009).

Pseudocapillaria tomentosa is a zebrafish pathogen that belongs to the Trichuridae family (the whipworms). Its relative *Trichuris trichiura* infects an estimated 1 billion humans and when it invades the gut epithelium it can lead to tissue damage, blood loss and anemia (Norhayati et al., 2003). However, the recent interest in treating inflammatory bowel disease with helminths, such as *Trichuris suis* ova (Weinstock and Elliott, 2013) makes the *P. tomentosa*/zebrafish model interesting from yet another perspective. How do these pathogens interact with and infect through epithelial surfaces? How do they pass through the blood–brain barrier? What roles do phagocytes play in restriction or promotion of infection? Zebrafish could be exploited to understand these and other disease-specific and general questions of immunity.

6.2. Why create experimental zebrafish models of human disease with human pathogens?

There are a number of questions about infectious disease that are practically difficult to address in the mouse or human host, but feasible in the zebrafish larval host (Tobin et al., 2012a). The transparency and genetic manipulability of the system enable high-resolution quantitative imaging to discover the mechanisms behind host and pathogen strategies. The use of zebrafish as a host for human pathogens allows for molecular genetic studies of the pathogen's virulence factors in conjunction with intravital imaging of immune responses.

What aspects of *in vivo* host–pathogen dynamics may be best addressed in the zebrafish? Recent infection studies with human pathogens in the zebrafish larva have highlighted some promising directions to pursue for a better understanding of immunity to and pathogenesis of eukaryotic pathogens (Meijer et al., 2013). What directs survival and proliferation of intracellular pathogens within immune and non-immune cell types (Roca and Ramakrishnan, 2013; Tobin et al., 2012b)? How is pathogen intracellular trafficking regulated among different compartments, and how is phagosome maturation manipulated by host and pathogen (Brothers et al., 2013; He et al., 2009; Mostowy et al., 2013; van der Vaart et al., 2012)? How do hosts and pathogens regulate the movement and dissemination of pathogens from one tissue to another (Clay et al., 2007)? What regulates the dynamics of immune recruitment and resolution during infection and sterile inflammation (Deng et al., 2013, 2011; Enyedi et al., 2013; Pase et al., 2012; Yang et al., 2012; Zakrzewska et al., 2010)? What are the roles of cytokines and factors in different tissues such as the vasculature and hematopoietic tissue (Elks et al., 2011; He et al., 2012; Mathias et al., 2006; Walters et al., 2010)? This broad set of questions comes from previous work in the zebrafish, but there is a much wider set of topics that will be applicable to this system.

7. Conclusion

Eukaryotic pathogens have been, are, and will continue to be of global importance in human and fish disease. Experimental infection has been effectively used to explore virulence of fungal and metazoan pathogens in the zebrafish, and can form the methodological basis for further expansion. There is tremendous potential to use the zebrafish system to address questions of both innate and adaptive immunity to eukaryotic pathogens. The imaging of transparent larvae and transparent adult Casper mutant zebrafish opens up both innate- and adaptive-focused studies that could address questions such as the conservation of T-helper subsets, the dynamics of fungal, protozoan and helminth interaction with epithelial and immune cells during infection, and the role of microbial colonization in immunity and disease.

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Abbreviations

HIV/AIDS	human immunodeficiency virus/acquired immune deficiency syndrome
PCP	<i>Pneumocystis</i> pneumonia
NADPH	nicotinamide adenine dinucleotide phosphate
Duox	dual-specific oxidase
Phox	phagocyte NADPH oxidase
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
TLR	toll-like receptor

CLR	C-type lectin receptor
IL	interleukin
Th	T helper
TALEN	transcription activator-like effector nuclease
CRISPR	clustered regularly interspaced short palindromic repeats

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Fig. 1.

Conservation of host cells and pathogen categories in zebrafish and mammalian infection by eukaryotes. The zebrafish is naturally infected by fungi, protozoans (microsporidian or dinoflagellate) and metazoans (myxozoan, cestodes or nematodes). In order to defend itself against pathogens, zebrafish use pathways conserved with mammals, with receptors such as TLRs or CLRs (Zhu et al., 2013) as well as signaling pathways and molecules such as those involved in myelopoiesis (Liongue et al., 2009), proinflammatory cytokines (Stockhammer et al., 2009), Th17 (Gunimaladevi et al., 2006) and Th2 type-cytokines (Balla et al., 2010; Zhu et al., 2012) found in both fish and mammals. The cells participating in the immune response are also similar, with chemotaxis of both neutrophils and macrophages to sites of fungal infection (Brothers et al., 2011) and activation of the NF- κ B pathway in epithelial cells in response to the presence of fungi. Dendritic cells (Wittamer et al., 2011), eosinophils (Balla et al., 2010), and mast cells (Dobson et al., 2008) are present in the juvenile zebrafish and eosinophils are able to respond to the presence of helminth secretory products (Balla et al., 2010). In the adult zebrafish, B and T cells promote a specific adaptive immune response (Page et al., 2013).

Table 1

Natural and experimental eukaryotic pathogens in zebrafish.

Single-celled eukaryotic pathogens			
Epidemiology	Pathogen class	Reference(s)	Pathogen(s)
Natural infection in zebrafish	Fungi (including Microsporidia)	<i>Pseudoloma neurophilia</i>	Cali et al. (2012), Matthews et al. (2001), Sanders et al. (2013)
		<i>Pleistophora hyphessobryconis</i>	Sanders et al. (2010)
		<i>Lecythophora mutabilis</i>	Dykstra et al. (2001)
	Dinoflagellate	<i>Piscinoodinium pillulare</i>	Paquette (2013)
	Ciliate	<i>Ichtyophthirius multifiliis</i>	Cherry (2003)
		<i>Paramecium caudatum</i> +	Peterson et al. (2013)
		<i>Mycobacterium marinum</i>	
	Oomycetes	<i>Tetrahymena</i>	Astrofsky et al. (2002)
		<i>Saprolegnia</i> spp.	Ke et al. (2009)
	Fungi	<i>Candida albicans</i>	Brothers et al. (2011), Chao et al. (2010)
Experimental infection of zebrafish with human pathogens		<i>Aspergillus fumigatus</i> (toxins)	Berthier et al. (2013)
		<i>Penicillium marneffei</i>	Ellett et al. (2011)
		<i>Giardia duodenalis</i>	Tysnes et al. (2012)
	Protozoan	Metazoan pathogens	
Epidemiology	Pathogen class	Pathogen(s)	Reference(s)
Natural infection in zebrafish	Nematode	<i>Pseudocapillaria tomentosa</i>	Balla et al. (2010), Kent et al. (2002)
	Cestodes	<i>Acanthostomum</i> spp., <i>Centrocestus</i> spp., et al.	Smith et al. (2011)
	Myxozoans	<i>Myxidium</i> spp.	Kent et al. (2012), Sanders et al. (2013)
Experimental infection of zebrafish with human pathogens	None		