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Zebra Fish in Tuberculosis Research

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Editorial

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Editorial

Mycobacterium

Tuberculosis (TB) is still the world's second deadliest infectious disease killing 1.5 million people and with an estimated 9.6 million new cases reported to the WHO in 2015. One-third of the world's population has been exposed to TB. 5-10% of these latent carriers will eventually develop the active disease [1]. A latent infection in granulomas has the ability to reactivate after many years, and the disease can be transmitted when granuloma integrity is lost. Primary TB mainly occurs in children, who are at the highest risk for TB meningitis and a disseminated form of the disease [2]. An alarming rise in antibiotic resistances and the lack of an effective vaccine against latent or reactivated TB emphasize the need for novel therapeutic strategies to control TB. As a live vaccine, BCG imposes a risk of a disseminated infection in immunocompromised patients [3]. Thus, there is a need to develop new effective drugs and vaccines against TB. For this purpose, relevant animal models are essential. The most commonly used animal models in TB research are mice, guinea pigs, and non-human primates (NHP), all of which have their limitations related to either space, costs, ethical aspects, or their ability to replicate the human disease pathology [4].

Mycobacterium Marinum

Mycobacterium marinum, is the causative agent of fish mycobacteriosis, and have a close relative of M. tuberculosis [5]. M. marinum spreads via water, and it also occasionally infects humans, but the infection is usually limited to the skin (fish tank granuloma) [6]. M. marinum is safer to work and has a shorter replication time than M. tuberculosis [7]. Similar to human TB, fish mycobacteriosis displays an acute and chronic form and the subsequently formed granulomas also resemble the lesions caused by M. tuberculosis. Both bacteria are having the ability to survive and replicate within macrophages [8]. In a laboratory setting, the zebrafish is an advantageous choice as a host organism for M. marinum for several reasons: multiple infection techniques can be used for both zebrafish embryos and adults [9].

The Zebrafish (Danio rerio)

Recently, the zebrafish-M. Marinum model has gained popularity as a natural pathogen–host system that closely recapitulates the pathology of human TB .The infection model and its applications are discussed in more detail below. The zebrafish is a tropical freshwater fish belonging to the minnow family (Cyprinidae) of the order Cypriniformes. Native to the Himalayan region, it is a popular aquarium fish, frequently sold under the trade name zebra danio [10]. The zebra fish is also an important and widely used vertebrate model organism in scientific research, and was among the first vertebrates to be cloned [11].

In Zebra Fish Embryos and Larvae

The external fertilization of zebrafish eggs provides easy access to developing embryos. Embryos naturally hatch by 2 days post fertilization (dpf), but the chorion can be removed at 1 dpf to facilitate experimental infection. By 72 h post fertilization (hpf), embryos reach the larval stage and larvae become capable of independent feeding by 5 dpf [12]. Pathology in zebrafish embryos is, because of practical/ethical reasons, usually only studied for 5–6 days. Within this short time frame early granuloma formation can be studied by real-time imaging .This allows visualization of early steps in mycobacterial pathogenesis in the context of innate

immunity. On infection, M. marinum is readily phagocytised by macrophages which traverse endothelial and epithelial barriers and form infectious clusters in deeper tissue within 4 days [13,14].

Adult Zebra Fish

While entry via de gastrointestinal tract is most likely the primary route of Mm infection in the natural environment, experimental infection of adult zebrafish is commonly achieved by intraperitoneal injection [15]. Dependent on the particular dose and strain, the infection manifests with acute symptoms or develops as a chronic progressive disease. Acute disease is characterized by rapid lethal inflammation and is more frequently observed with human-derived isolates of Mm that form a distinct genetic cluster [16]. Swelling of the abdomen, haemorrhages, and skin ulcerations are typically observed at the end stage of the chronic progressive disease. This is associated with a strong induction of immune response genes and inflammation markers at the transcriptional level [17]. Well before external symptoms become apparent, well-organized granulomas are formed in different organs, including the liver, pancreas, kidney, intestines, and spleen and sometimes also in the connective tissues [18]. Some intraperitoneally infected zebrafish also develop granulomas in close relation with brain tissue and meninges; therefore, the model can also be used to study TB meningitis [19].

Beyond the larvae, adult zebrafish infections have enabled researchers to investigate mycobacterial infection in the context of both the innate and adaptive immune systems. Low-dose infection of adult zebrafish with the Aronson strain of M. marinum leads to persistent infections resembling latency that can be reactivated by immunocompromise [20]. Researchers taking advantage of the unique benefits of the zebrafish–M. Marinum system have been able to answer longstanding questions and open new fields of inquiry regarding innate immunity, granuloma dynamics, and host and bacterial genetics within mycobacterial pathogenesis.

The study of mechanisms underlying latency and reactivation of TB is hampered by the limitations of animal models. Recently, it has been shown that the zebrafish-Mm model can be used to mimic aspects of latent disease [21]. Several weeks after intraperitoneal injection with a low-dose of Mm bacteria, zebrafish developed stable bacterial loads and constant numbers of granulomas. Ex vivo activation by resuscitation promoting factor demonstrated the dormancy of Mm under these conditions. The development of latency relies on rag1-mediated adaptive immunity, and immunosuppression induced by gamma irradiation leads to reactivation of the dormant bacterial population. This model has much potential for preclinical testing of new drug and vaccine candidates.

Antimycobacterial Compounds Screening

New antimicrobial compounds or therapies can be accelerated using the zebrafish model. Mainly activity and dosage of antimycobacterial compounds in zebrafish closely resemble characteristics in humans [22]. In addition, the zebrafish model has helped to challenge the model that persistence is linked to arrested growth [23]. Using the zebrafish model, it was shown, by spatial monitoring of the behaviour of fluorescent bacteria after treatment with antibiotics, that both Macrophages and granulomas play a role in the induction and dissemination of drug-tolerant bacteria. The intramacrophage-mediated oxidative stress induces the expression of bacterial efflux pumps in actively replicating bacteria. It was also shown that bacterial efflux.

Pathogenesis of Mycobacterium sps

The embryo of a zebra fish is an excellent model to study the importance of mycobacterial virulence factors in different steps of infection. The first identified erp (pirG) gene, coding for a cell wall–associated protein with unknown function, as required for the virulence in M. tuberculosis [24]. Using microscopic examination of infected zebrafish embryos it could be shown that *M. marinum* lacking Erp failed to grow and survive upon phagocytosis, an event very early in granuloma pathogenesis [25]. Macrophages were eliminated in zebrafish embryos by injection of pu.1 morpholino, thereby knocking down the pu.1 transcription factor, which is required for myeloid development [25]. A number of studies have used different setups to identify M. marinum virulence factors, most of which seem to underscore the similarities between M. marinum and M. tuberculosis.

In conclusion zebrafish model has many inherent advantages for the study of tuberculosis pathogenesis. Zebrafish naturally develop a tuberculosis-like disease upon infection with Mm, enabling us to examine a multi-layered immune response that has developed over evolutionary timescales. The fish is also cheap, fecund, genetically tractable, and optically transparent, facilitating real-time non-invasive microscopy. Studies in the zebrafish have changed how we think about

tuberculosis, teaching us that the granuloma is dynamic, mycobacteria become tolerant to antibiotics by inducing expression of efflux pumps, and that both inadequate and hyper inflammatory states are pathological. The zebrafish model is likely to continue making important inroads into understanding the confusing and often contradictory clinical realities of tuberculosis.

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