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Short communication

Mycobacterium marinum infection drives foam cell differentiation in zebrafish infection models

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ABSTRACT

Host lipid metabolism is an important target for subversion by pathogenic mycobacteria such as *Mycobacterium tuberculosis*. The appearance of foam cells within the granuloma are well-characterised effects of chronic tuberculosis. The zebrafish-*Mycobacterium marinum* infection model recapitulates many aspects of human-M. tuberculosis infection and is used as a model to investigate the structural components of the mycobacterial granuloma. Here, we demonstrate that the zebrafish-*M. marinum* granuloma contains foam cells and that the transdifferentiation of macrophages into foam cells is driven by the mycobacterial ESX1 pathogenicity locus. This report demonstrates conservation of an important aspect of mycobacterial infection across species.

1. Introduction

Studies focused on the important human pathogen *Mycobacterium tuberculosis* (*Mtb*) have identified direct and indirect mycobacterial metabolism of host lipids, and the transformation of macrophages into foam cells, as important pathways in mycobacterial pathogenesis (Lovewell et al., 2016). During early stages of granuloma formation in Mtb infection, macrophage transformation into foamy macrophages is driven by the internalisation of low-density lipoprotein particles and the retention of esterified cholesterol in the form of lipid droplets (Cardona et al., 2009). Foamy macrophages have been identified as an important nutrient source sustaining *Mtb* during infection. Additionally, foamy macrophages have been implicated in inhibiting lymphocyte access to infected macrophages and the build-up of caseum at the centre of granulomas, resulting in granuloma breakdown (Dong et al., 2018; Pandey and Sassetti, 2008; Russell et al., 2009).

The zebrafish (*Danio rerio*) model is a powerful platform for the investigation of host-pathogen interactions. Due to their optical transparency, the zebrafish larval infection model facilitates multiday observation of mycobacterial pathogenesis during early stages of infection in real-time within a live vertebrate (Oehlers et al., 2015). Moreover, the larval zebrafish immune system offers the ability to observe bacterial interaction with an innate immune system, highly conserved with mammals (Matty et al., 2016). Use of the zebrafish-*M. marinum* infection model has uncovered key insights into the granuloma as a bacterially-driven haven supporting tuberculosis pathogenesis (Cronan et al., 2016; Davis and Ramakrishnan, 2009). However, little is known about conservation of altered host lipid metabolism as a conserved motif across mycobacterial infections.

2. Materials and methods

2.1. Zebrafish handling

Adult zebrafish were housed at the Garvan Institute of Medical Research Biological Testing Facility (St Vincent's Hospital AEC Approval 1511) and housed for infection experiments at the Centenary Institute (Sydney Local Health District AEC Approval, 2016-037). Zebrafish embryos were obtained by natural spawning and embryos were raised at 28 °C in E3 media. All experiments and procedures were completed in accordance with Sydney Local Health District animal ethics guidelines for zebrafish embryo research.

2.2. Infection of adult zebrafish

Adult zebrafish, between the ages of 3 months and 12 months, were

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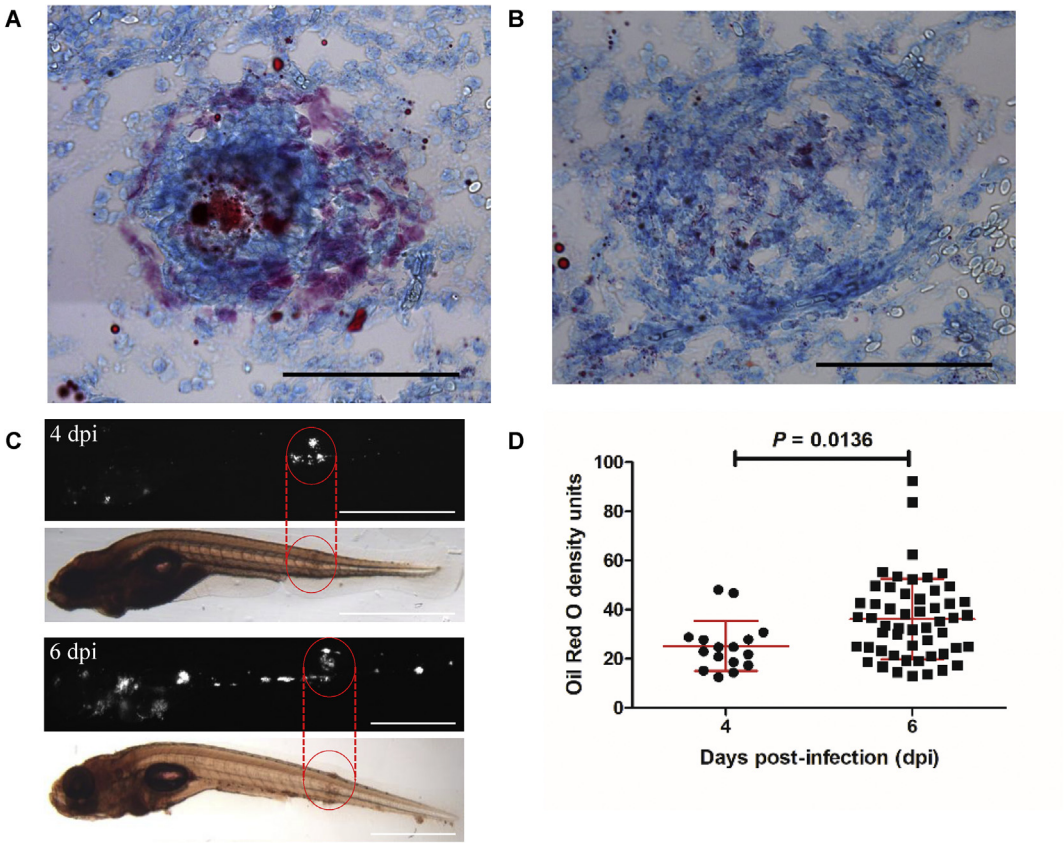

Fig. 1. Lipid accumulation is present within the granulomas of zebrafish adults and embryos infected with *M. marinum*.
A, Oil Red O staining of a compact, presumptively necrotic, granuloma from an adult zebrafish infected with *M. marinum* at 2 weeks post-infection. Images are representative of granulomas identified from 3 animals. *B,* Oil Red O staining of a loose, presumptively cellular, granuloma from an adult zebrafish infected with *M. marinum* at 2 weeks post-infection. Note the heavier Oil Red O staining in the necrotic granuloma. Scale bars represent 100 μm. *C,* Representative images of Oil Red O staining density at 4 and 6 days post-infection from experimental dataset in 1D. Red circles indicate areas of embryos with largest foci of infection. Scale bars represent 500 μm. *D,* Quantification of Oil Red O staining density of granulomas in zebrafish embryos at 4 and 6 days post-infection. Each data point represents the Oil Red O staining density of an individual granuloma. Error bars represent standard deviation, statistical tests were performed using an unpaired T-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

infected with approximately 200 CFU M. *marinum* by intraperitoneal injection as previously described (Oehlers et al., 2015). Infected animals were recovered into 1 g/L salt water, then fed and monitored daily.

2.3. Histological processing of adult zebrafish for Oil Red O staining

Adult zebrafish were euthanized by anesthetic overdose at 2 weeks post-infection and fixed in 10% neutral buffered formalin for 2 days at 4 °C. Fixed specimens were washed in PBS, 30% (w/v) sucrose, 50:50 solution of 30% sucrose and OCT, and a final wash in OCT before freezing. Tissue sections were cut at 20 μm on a Leica cryostat. Slides were re-fixed in 10% neutral buffered formalin, rinsed in propylene glycol, stained in 0.5% (w/v) Oil Red O dissolved in propylene glycol and counterstained with a 1% (w/v) solution of methylene blue.

2.4. Infection of zebrafish embryos

Zebrafish embryos were raised to 30–48 h post fertilization and anaesthetised with 160 μg/mL tricaine (Sigma-Aldrich) prior to infection with approximately 200 CFU M strain *M. marinum* via caudal vein injection. A dose of approximately 1000 CFU Δ*ESX1 M. marinum* was injected to match parental strain burden at 5 dpi. Embryos were recovered into E3 supplemented with phenylthiourea.

2.5. Oil Red O staining

Oil Red O lipid staining on whole mount embryos was completed as previously described (Passeri et al., 2009). Briefly, embryos were individually imaged for bacterial distribution by fluorescent microscopy, fixed, and stained in Oil Red O (0.5% w/v in propylene glycol). Oil Red O staining intensity at sites of infection were quantified in ImageJ and calculated as the pixel density difference from uninfected tissue.

2.6. Imaging

Live zebrafish embryos were anaesthetised in tricaine and mounted in 3% methylcellulose for imaging on a Leica M205FA fluorescence stereomicroscope. Histological sections were imaged on a Leica DM6000B. Further image manipulation and/or bacterial quantification was carried out with Image J Software Version 1.51j.

2.7. Quantification of M. marinum burden by fluorescent pixel count

Infection burden was measured as the number of pixels in each embryo above background fluorescence in ImageJ (National Institutes of Health) and pixels counted using the 'Analyse particles' function (Matty et al., 2016).