Analysis of mycobacterial infection-induced changes to host lipid metabolism in a zebrasfish infection model reveals a conserved role for LDLR in infection susceptibility

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Abstract – 250 words

Host lipid metabolism is an important target for subversion by pathogenic mycobacteria such as Mycobacterium tuberculosis. Systemic dyslipidemia and the appearance of foam cells within the granuloma are well-characterised effects of chronic tuberculosis. The zebrafish-M. marinum infection model recapitulates many aspects of human-M. tuberculosis infection and is used as model to investigate the structural components of the mycobacterial granuloma. Here, we demonstrate that the zebrafish-M. marinum granuloma contains foam cells and that transdifferentiation of macrophages into foam cells is driven by the mycobacterial ESX1 pathogenicity locus. Furthermore, we define a detrimental role for infection-induced alterations host lipid metabolism in mycobacterial pathogenesis. Depletion of LDLR, a key lipid metabolism node, decreased M. marinum burden, and corrected infection-induced altered lipid metabolism resulting in decreased LDL and the reduced rate of
macrophage transformation into foam cells. Our results demonstrate a conserved role for host lipid metabolism, and specifically the LDL-LDLR axis, in mycobacterial pathogenesis.

Importance – 150 words
Mycobacterial infections are classically defined by the presence of granulomas at the site of infection. Foams cells are a key component of mycobacterial granulomas and arise as a consequence of local and systemic changes to host lipid metabolism. It is hypothesised that foam cells provide a lipid-rich intracellular environment, promoting mycobacterial survival within the granuloma. Here we have turned to the zebrafish-\textit{M. marinum} platform to identify the host-pathogen interactions contributing to macrophage transdifferentiation into foam cells and the consequences of infection-induced dyslipidemia. We observe foam cells in embryonic and adult zebrafish granulomas, and identify a role for the mycobacterial ESX1 pathogenicity locus in promoting foam cell differentiation. We show that zebrafish-\textit{M. marinum} infection results in dysregulation of host lipid metabolism, and that depletion of the host lipid metabolism node LDLR corrects infection-induced changes to host lipid metabolism and reduces foam cell formation.

Introduction
Mycobacterial manipulation of host lipid metabolism and the transformation of macrophages into foam cells are important pathways in the pathogenesis of \textit{Mycobacterium tuberculosis (Mtb)} (1). Mycobacteria drive the transformation of infected macrophages into foam cells through the imbalance of low density lipoprotein (LDL) influx and efflux mechanisms (2). Given the significant proportion of the mycobacterial genome that is allocated to lipid metabolism, and the utilisation of lipids such as cholesterol for growth \textit{in vitro}, the abundance
of neutral lipids contained within the harsh granuloma microenvironment may supply a
50   carbon source for intracellular mycobacterial survival and persistence (3).
51
Dyslipidaemia and foam cell differentiation are known to contribute to disease pathology and
53   prognosis in human Mtb infection where foamy macrophages may inhibit lymphocyte access
54   to infected macrophages, increase the build-up of caseum at the center of granulomas, and
55   provide lipids utilized during evasion of phagocytosis (4, 5). However, the involvement of
56   foam cells and dyslipidemia in the pathogenesis of non-tubercular mycobacterial infections
57   such as Mycobacterium avium subspecies paratuberculosis (MAP) or Mycobacterium
58   marinum has not been explored. Our group has identified infection-induced changes to host
59   lipid metabolism genes in MAP-infected cattle and aberrant host lipid metabolism gene
60   expression and intracellular cholesterol accumulation within MAP-infected macrophages (6,
61   7). Here, we have turned to the natural host-pathogen pairing of the zebrafish-M. marinum
62   platform to investigate the conservation and consequences of altered host lipid metabolism as
63   a conserved motif across mycobacterial infections.
64
Zebrafish (Danio rerio) are a powerful animal model to study early host-pathogen
66   interactions. Zebrafish demonstrate a functional innate immune system from early in
67   embryogenesis with a high degree of homology to mammals (8). M. marinum is a natural
68   pathogen of fish and amphibian species and genomic analysis has identified M. marinum as
69   the closest genetic relative of the Mtb complex, sharing 85% of orthologous regions with Mtb
70   (9). Zebrafish models of M. marinum infection have provided unique insight into host-
71   pathogen interplay responsible for granuloma formation, exquisitely reproducing the cellular
72   structure of the human-M. tuberculosis granulomas (10-12). As such, the zebrafish-M.
73   marinum model is an important platform for investigating immunopathogenesis of the
mycobacterial granuloma, providing fundamental insights into the role of macrophage epithelial transdifferentiation in granuloma maturation (10).

The aim of this study was to explore the biological significance of infection-induced lipid metabolism in a model non-tubercular mycobacterial infection. Our findings demonstrate the key lipid metabolism node Low Density Lipoprotein Receptor (LDLR) mediates the uptake of lipids into the mycobacterial granuloma niche and aids the transformation of macrophages into foam cells.

Results

Lipid accumulation is observed in adult zebrafish-\textit{M. marinum} granulomas

Adult zebrafish infected with \textit{M. marinum} form highly organized and caseous necrotic granulomas that recapitulate many important aspects of the human-\textit{M. tuberculosis} granuloma (10, 11). To determine if lipids accumulated in these complex zebrafish-\textit{M. marinum} granulomas, adult zebrafish (>3 months old) were infected by intraperitoneal injection of \textit{M. marinum} and maintained for 2 weeks post-infection, a time point where the animals contain a mixture of cellular and necrotic granulomas (11, 12). Strong Oil Red O staining was observed in the cellular layer of necrotic granulomas and weaker Oil Red O staining was observed throughout cellular granulomas (Figure 1A and 1B).

The finding that detectable foam cells correlate with granuloma maturation is consistent with their hypothesized role in granuloma immunopathology (4). We hypothesize cellular granulomas with weak lipid accumulation mature into necrotic granulomas with heavier lipid accumulation. To investigate this hypothesis, we switched model system to the more
experimentally amenable zebrafish embryo to characterize the genesis of foam cells through temporal and functional analyses.

**Lipid accumulation is observed in zebrafish embryo-** *M. marinum* **granulomas**

Zebrafish embryos lack an adaptive immune system and form simple *M. marinum*-containing granulomas primarily consisting of macrophages (10). These simple granulomas have demonstrated remarkable immunopathology fidelity to more complicated adult zebrafish and mammalian granulomas (10-13). We investigated the temporal relationship of foam cell formation with known stages of granuloma maturation in the embryo model. Embryos were individually imaged for fluorescent mycobacterial distribution and stained with Oil Red O at 4 and 6 days post-infection (dpi). Granuloma lipid accumulation was first observed at 4 dpi and was increased in intensity at 6 dpi (Figures 1C and 1D).

Consistent with the Oil Red O staining pattern in adult granulomas, the appearance of putative foam cells in the embryo granulomas correlated with granuloma maturation and was markedly increased at a timepoint when there is significant granuloma necrosis.

**Foam cell formation is driven by mycobacterial pathogenesis**

To establish if *M. marinum* drives foam cell transdifferentiation as a part of its granuloma-inducing pathogenic program, we infected zebrafish embryos with ∆ESX1 *M. marinum*. The ∆ESX1 mutant strain is able to persist and multiply in zebrafish embryos, however it drives granuloma maturation to a much lesser degree than the parental strain (13, 14). Compared to burden-matched embryos infected with WT *M. marinum*, ∆ESX1-infected larvae showed significantly less Oil Red O staining in mycobacterial granulomas (Figures 2A and 2B).
This experiment suggests foam cell genesis is driven by pathogenic mycobacteria as part of the ESX-1 virulence program. Taken with the circumstantial evidence that foam cells provide a niche for mycobacterial growth, and a haven from antibiotics and immune killing, this data suggests mycobacteria manipulate host lipid metabolism to drive transdifferentiation of permissive foam cells.

Infection-induced altered host dislipidemia is recapitulated in the zebrafish-\textit{M. marinum} infection model

Previous reports have identified hypercholesterolemia as a correlate of disease pathology during human \textit{Mtb} infection (5). To determine whether these findings were conserved within the zebrafish-\textit{M. marinum} platform, we infected zebrafish embryos with \textit{M. marinum} then analysed the total embryo LDL and HDL content at 3 and 5 dpi, corresponding to an early infection timepoint and the onset of granuloma necrosis, respectively. Calculation of lipoprotein content revealed a skewing towards LDL demonstrating conservation of the infection-induced dyslipidemia phenotype in our zebrafish embryo model (Figure 3A).

Knockdown of \textit{Ldlra} reduces \textit{M. marinum} burden in zebrafish embryos

Infection of macrophages with various species of mycobacteria, including in the zebrafish-\textit{M. marinum} system, increases \textit{LDLR} transcription (6, 15). Given the infection-induced changes in the ligand LDL in our model, we tested the functional relevance of \textit{LDLR} by morpholino knockdown of the zebrafish \textit{ldlra} gene (16). Knockdown of \textit{Ldlra} had no effect on embryo development and editing of the \textit{ldlra} transcript was observed (Figures 3B, S1A and S1B). Oil Red O staining for lipids in \textit{Ldlra}-depleted embryos revealed marked vascular lipid accumulation consistent with previous reports of \textit{Ldlra} depletion being atherogenic (Figure S1C) (16).
Following Ldlra-depletion, embryos were infected with fluorescent *M. marinum* and bacterial burden was analysed by fluorometry. Zebrafish embryos depleted of Ldlra displayed a significantly decreased bacterial burden at 3 and 5 dpi when compared to control embryos (Figures 3C, 3D, S2A and S2B).

**Knockdown of Ldlra corrects infection-induced altered host lipid metabolism**

Host lipid metabolism is a tightly controlled biological pathway, responsible for addressing the cellular lipid requirements through the regulation of influx and efflux mechanisms. Of the many surface receptors involved in lipid metabolism, LDLR is arguably the most important, playing a key role in driving the uptake of circulating LDL (2).

Following *M. marinum* infection, expression of the LDLR-network and lipid efflux gene *ABCA1* was markedly increased (Figure 4A). Depletion of Ldlra significantly reduced *abca1* transcription, demonstrating a transcriptional conservation of infection-induced lipid metabolism network in our model (Figure 4A).

We hypothesised that infection-induced LDLR is a key node in driving infection-induced hyperlipidaemia, and that depletion of LDLR would prevent infection-induced hypercholesterolemia and the accumulation of lipids within granulomas. Following *M. marinum* infection, Ldlra-deficient embryos displayed a decreased LDL:HDL ratio compared to infected controls at 5 dpi (Figure 4B).

Next, we hypothesised that the combination of reduced potential for LDL uptake and correction of total cholesterol levels would reduce granuloma lipid accumulation in Ldlra-
depleted embryos. Granuloma Oil Red O lipid staining density was reduced in infected Ldlra-
deficient embryos compared to infected controls demonstrating a functional role for LDLR in
directing bacterial-beneficial lipid accumulation during mycobacterial infection (Figures 4C,
4D, S3A and S3B).

Discussion

A hallmark of mycobacterial disease is the formation of granulomas at the site of infection
(17). Foam cells are an important histological component of granulomas and their
transformation is driven by the dysregulation of macrophage lipoprotein influx and efflux (2).
These lipid-filled macrophages are deficient in their phagocytic abilities and provide an
intracellular carbon-based nutrition source, enabling the intracellular survival of
mycobacteria (2). Our findings in this study demonstrate 1) infection-induced altered host
lipid metabolism is a conserved feature of non-tuberculous mycobacterial infection, and 2) host expression of LDLR is co-opted by mycobacterial infection to increase susceptibility to
mycobacterial infection.

This is the first report of mycobacterial infection-induced foam cells in the zebrafish model
and recapitulates an important histological correlate of human TB disease in this important
model organism. Our results show foam cell transdifferentiation is a correlate of granuloma
maturation and that this accumulation of lipids in the granuloma is part of a highly conserved
mycobacterial pathogenicity program. Our findings demonstrate the zebrafish-\textit{M. marinum}
platform will be applicable further mechanistic studies of infection-induced foam cell
transdifferentiation.
The elevation of host lipids following mycobacterial infection has been well documented in both human and murine *Mtb* infections (5, 18). Recently, we have shown that elevated serum cholesterol is associated with histopathological lesions in sheep and cattle infected with MAP (19). The results from the current study are in agreement with previous findings and suggest that the induction of host hypercholesterolemia is a conserved motif of pathogenic mycobacteria. Furthermore, our results demonstrated that *LDLR* knockdown significantly altered the availability of circulating lipoproteins and decreased Oil Red O staining density, providing strong evidence to suggest that the abundance of neutral lipids and therefore foam cells, was largely decreased as a consequence of *LDLR* depletion.

Host lipid metabolism is a complex pathway involving many transcription factors, surface molecules and feedback mechanisms. In the absence of key components, compensatory pathways are utilised to achieve cellular homeostasis. Expression of *abca1* was increased following *M. marinum* infection, in agreement with previous studies identifying the upregulation of *ABCA1* by mycobacterial infection across multiple host species (20). Interestingly, there was a notably lower expression in *ldlra*-deficient embryos, presumably due to the decreased efflux of lipids from mycobacteria-infected macrophages as a consequence of the altered lipoprotein abundance.

Collectively, this study provides evidence of conserved infection-induced altered host lipid metabolism in the zebrafish-*M. marinum* model, and highlights a crucial role of *LDLR* in infection susceptibility across host and pathogen species.

**Materials and Methods**

**Zebrafish maintenance and breeding**
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.

**Morpholino design and microinjection**

Splice-blocking morpholino oligonucleotide for *LDLR* (gene variant *ldlra*) was designed from Ensembl transcript sequences ([http://www.ensembl.org/index.html](http://www.ensembl.org/index.html)) and purchased from Gene Tools. The *LDLR* morpholino (5’-ATCACATTTCATTTCTTACAGT-3’) was targeted at the exon 4/intron 4 and 5 splice junction that was expected to cause the excision of exon 4. The control morpholino (5’-CCTCTTACCTCAGTTACAATTTATA-3’) is a negative control targeting a human beta-globin intron known to cause little phenotype variations in zebrafish.

Embryos at the 1-4 cell stage were injected with 2 nL of morpholino into the yolk. Embryos were raised at 28°C in E3 media.

**Infection of adult zebrafish**

Adult zebrafish, between the ages of 3 months and 12 months, were infected with approximately 200 CFU *M. marinum* by intraperitoneal injection as previously described (11). Infected animals were recovered into 1 g/L salt water, then fed and monitored daily.

**Histological processing of adult zebrafish**

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. 20 microns sections were cut on a Leica cryostat. Slides were refixed 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.

**Infection of zebrafish embryos**

At 30 hours post-fertilisation, zebrafish embryos were anaesthetised with 160 µg/mL tricaine (Sigma-Aldrich) and infected with approximately 200 CFU M strain *M. marinum* via caudal vein injection. A dose of approximately 1000 CFU \(\Delta ESX1\) *M. marinum* was injected to match parental strain burden at 5 dpi.

**Oil Red O staining**

Oil Red O lipid staining on whole mount embryos was completed as previously described (21). 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 above background tissue.

**Imaging**

Live zebrafish embryos were anaesthetized in M-222 (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.

**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 (22).

Lipoprotein assay

Lipoprotein abundance was measured using the HDL & LDL/VLDL Cholesterol Assay Kit (Cell Biolabs) as per manufacturer’s instructions and based on previously published methods (16). Briefly, 10 embryos per treatment group were terminally anaesthetised and placed into BHT solution (10 µg/mL, Sigma-Aldrich) and homogenised. Homogenates were centrifuged at 4°C at 2000 x g for 5 mins to pellet debris. The supernatant was mixed with an equal volume of precipitation solution to separate the high density lipoprotein (HDL) and low density lipoprotein (LDL) fractions. Each lipoprotein fraction was mixed with an equal volume of reaction mix and quantified by fluorescent plate reader. A standard curve was included in each run to calculate the concentration of lipoproteins within each treatment.

Molecular analysis of ldlra knockdown

RNA was extracted in TRIZOL (Thermofisher) by precipitation and cDNA was synthesised with an Applied Biosystems High Capacity cDNA reverse transcription kit (Thermofisher). PCR was performed with ldlra-specific primers (5’-AGAGCTGGAAATGTGACGGA-3’ and 3’-CTCATCTGGACGGCATGTTG-5’) and visualised by gel electrophoresis.

Quantitative PCR

Quantitative PCR (qPCR) reactions were completed as previously described (23) using Mx3000P real-time PCR systems (Stratagene). Gene-specific primers were generated for abca1 (5’-AGCTGCTGGTGACGGCATGTTGGA-3’ and 3’-CTGTCTCAGTCATGTTG-5’).
for comparison against housekeeping gene actb (5’-CCTTCCAGCAGATGTGGATT-3’ and
3’-CACCTTCACCGTTCCAGTTT-5’). Samples were run in duplicate with reaction
specificity confirmed based on the post-amplification dissociation curve.

Statistical analysis

Genstat (VSN International) and Prism (Graphpad) were used to perform statistical testing as
indicated. Bacterial burden was examined using Restricted Maximum Likelihood (REML)
coupled with Fishers protected LSD; experiment number was included as the random effect
to account for variability between experimental replicates. Lipoprotein abundance, Oil Red O
staining density and gene expression analysis were analysed using unpaired Student’s t-tests.

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References


human tuberculosis granulomas correlates with elevated host lipid metabolism.

EMBO Molecular Medicine 2:258-74.


23. Taylor DL, Zhong L, Begg DJ, de Silva K, Whittington RJ. 2008. Toll-like receptor genes are differentially expressed at the sites of infection during the progression of
Johne's disease in outbred sheep. Veterinary Immunology and Immunopathology

124:132-151.
Figure Legends

Figure 1: Lipid accumulation is present within the granulomas of zebrafish adults and embryos infected with *M. marinum*.

A, Oil Red O staining of a necrotic granuloma from an adult zebrafish infected with *M. marinum* at 2 weeks post-infection. B, Oil Red O staining of a 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. 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.

Figure 2: Granuloma lipid accumulation is driven by mycobacterial pathogenicity locus ESX1.

A, Representative images of Oil Red O staining in zebrafish embryos infected with wild-type or ΔESX1 *M. marinum* at 5 days post-infection. Red circles indicate areas of embryos with largest foci of infection. Scale bars represent 500 µm. B, Quantification of Oil Red O density units in embryos infected with wild-type or ΔESX1 *M. marinum*. Each data point represents an individual granuloma. Error bars represent standard deviation. Analysis of Oil Red O density units was completed using an unpaired Student’s *t*-test.

Figure 3: LDL is increased by infection and knockdown of Ldlra reduces *M. marinum* bacterial burden.
A, Quantification of LDL concentration in wild-type zebrafish embryos at 5 days following *M. marinum* infection. Each data point represents a pooled sample of 10 embryos. B, Molecular validation of *ldlra* morpholino efficacy at 3 and 5 days post-treatment. C, Representative images of *M. marinum*-wasabi bacterial distribution at 5 days-post infection. Scale bar represents 500 µm. D, Quantification of *M. marinum* bacterial burden by fluorometry at 5 days post-infection. Error bars represent standard deviations. Each data point represents an individual embryo. Statistical tests for LDL quantification were performed using unpaired Student’s *t*-tests. Analysis of bacterial burden was performed using an unpaired Student’s *t*-test. Experiments were completed with 4 independent biological replicates for each panel.

Figure 4: Mycobacterial infection-induced modulation of host lipid metabolism is corrected in *Ldlra*-deficient embryos.

A, *abca1* transcript fold change expression at 5 days post-infection measured by qPCR. Each column represents two independent biological replicates completed with technical replicates. B, Quantification of LDL concentration in *Ldlra*-deficient embryos at 5 days-post infection. Each data point represents a pooled sample of 10 embryos completed with four independent replicates. C, Representative images of Oil Red O staining density at 5 days post-infection. Red circles indicate areas of embryos with largest foci of infection. Scale bar represents 500 µm. D, Quantification of granuloma Oil Red O staining density units at 5 days post-infection. Each data point represents an individual granuloma. Error bars represent standard deviation. Gene expression analysis was completed using one-way ANOVA. Analysis of LDL concentration and Oil Red O density units was completed using an unpaired Student’s *t*-test.
Supplementary figure 1: Knockdown of Ldlra results in extensive lipid accumulation with the vasculature. 

A, Normal morphology and phenotypes at 5 days post-fertilisation. B, Embryos deficient in Ldlra show normal phenotypes at 5 days following morpholino treatment. C, Embryos deficient in Ldlra display visible lipid abundance within the vasculature following Oil Red O lipid staining. White arrows represent expansive lipid accumulation within the vasculature. Scale bars represent 500 µm.

Supplementary figure 2: Bacterial burden is reduced in Ldlra-deficient embryos at 3 days post-infection. 

A, Representative images of M. marinum bacterial burden at 3 days-post infection. Scale bars represent 500 µm. Quantification of M. marinum bacterial burden at 3 days post-infection. Error bars represent standard deviations. Each data point represents an individual embryo. Analysis of bacterial burden was performed using an unpaired Student’s t-test. Experiments were completed with 4 independent biological replicates for each panel.

Supplementary figure 3: Granuloma Oil Red O staining is reduced in Ldlra-deficient embryos at 3 days post-infection with M. marinum. 

A, Representative images of Oil Red O staining density at 3 days post-infection with M. marinum. Scale bars represent 500 µm. B, Quantification of Oil Red O density units at 3 days post-infection with M. marinum. Each data point represents an individual granuloma. Error bars represent standard deviation. Statistical tests were performed using an unpaired Student’s t-test.
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A. Representative images of *M. marinum* bacterial burden at 3 days-post infection. Scale bars represent 500 µm.

Quantification of *M. marinum* bacterial burden at 3 days post-infection. Error bars represent standard deviations.

Analysis of *M. marinum* bacterial burden was completed using REML coupled with Fisher’s protected LSD; experiment number was included as random effect.
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