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J Immunol 2019; 202:494-502; Prepublished online 14 December 2018;
doi: 10.4049/jimmunol.1801139
http://www.jimmunol.org/content/202/2/494

Supplementary Material
http://www.jimmunol.org/content/suppl/2018/12/14/jimmunol.1801139.DCSupplemental

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Drug-resistant mycobacteria are a rising problem worldwide. There is an urgent need to understand the immune response to tuberculosis to identify host targets that, if targeted therapeutically, could be used to tackle these currently untreatable infections. In this study we use an Il-1β fluorescent transgenic line to show that there is an early innate immune proinflammatory response to well-established zebrafish models of inflammation and Mycobacterium marinum infection. We demonstrate that host-derived hypoxia signaling, mediated by the Hif-1α transcription factor, can prime macrophages with increased levels of Il-1β in the absence of infection, upregulating neutrophil antimicrobial NO production, leading to greater protection against infection. Our data link Hif-1α to proinflammatory macrophage Il-1β transcription in vivo during early mycobacterial infection and importantly highlight a host protective mechanism, via antimicrobial NO, that decreases disease outcomes and that could be targeted therapeutically to stimulate the innate immune response to better deal with infections. The Journal of Immunology, 2019, 202: 494–502.

Pulmonary tuberculosis (TB) is a major world health problem caused by the bacillus Mycobacterium tuberculosis (1). It is a current priority for infectious disease research because of increasing rates of multi- and totally drug-resistant strains causing high levels of mortality, especially in the immunocompromised (2). Mycobacteria are specialized at evading killing mechanisms of the immune system to survive. Mycobacteria and immune cells create a highly organized niche, called the granuloma, in which M. tuberculosis can proliferate or enter a latent phase, protected from the immune system (3, 4). In human M. tuberculosis infection, bacteria first encounter cells of the innate immune system in and around the lungs, either macrophages in the alveolar space or neutrophils in the surrounding capillary vasculature, before the involvement of adaptive immunity and granuloma formation (5, 6). These initial phagocytosis events are followed by the attraction of other innate immune cells which signal to draining lymph nodes to activate the adaptive immune response, signs of which only become apparent 3–8 weeks post-infection in humans (6). Although granuloma formation is reasonably well characterized, the initial interactions of the bacteria with the host innate immune cells are less well defined in vivo.

M. tuberculosis, like many other bacterial and pathogenic microbes, triggers a proinflammatory immune response via the activation of TLRs (7). The activation of the innate immune cells via TLR signaling is a critical early host response to many invading pathogens for successful clearance of infection, and, in the absence of TLR signaling, mycobacteria grow unchecked to cause systemic infection (8). Although mycobacteria can hijack host leukocytes to create a niche for their growth, in zebrafish models, many of the initial M. marinum inoculum are neutralized by macrophages and neutrophils before infection can take hold (9, 10). Early mycobacterial interaction with host leukocytes is critical for the pathogen, and manipulation of the macrophage by the bacteria is required for establishment of a permissive niche in which the bacteria can grow and build its host-derived protective structure, the granuloma (11, 12). Indeed, the control of the macrophage by M. marinum may happen early in infection, as there is a phase of infection from 6 h to 1 d postinfection (dpi) in the zebrafish model that is characterized by a dampening of the cytokine transcriptional response (13). Greater understanding of the diverse phenotype of macrophages immediately postinfection may allow therapeutic tuning to provide maximal early control of mycobacteria during infection (14, 15). Recent studies in optically translucent zebrafish infection models have indicated that initial interactions between M. marinum and macrophages and neutrophils are more complex than originally thought, with successive rounds of bacterial internalization and leukocyte cell death leading to granuloma formation (9, 16, 17). The immune molecular...
mechanisms involved in these early processes are poorly understood.

We have previously demonstrated in a zebrafish/M. marinum model of TB that the initial immune response to infection can be enhanced by stabilizing host-derived hypoxia-inducible factor-1α (Hif-1α), leading to reduced bacterial burden (18). Hif-1α is a major transcriptional regulator of the cellular response to hypoxia, which has been implicated in the activation of macrophages and neutrophils during infection and inflammatory processes (19, 20). Stabilization of Hif-1α in zebrafish upregulated proinflammatory neutrophil NO production, leading to lower mycobacterial burden (18, 21). The mechanisms by which proinflammatory cytokines associated with this NO increase are regulated by Hif-1α signaling is not known.

II-1β is a critical macrophage-derived activator of immune cells with wide-ranging and complex effects on immune signaling and downstream pathways. II-1β has been shown to be upregulated in the onset and formation of M. marinum and M. tuberculosis granulomas (22–24). We hypothesized that II-1β would be activated in specific immune cell populations early in M. marinum infection (within 1 dpi, pregranuloma formation) and that Hif-1α acts via altered expression of this important proinflammatory mediator to confer protection against mycobacterial infection. In this study, using the zebrafish M. marinum model and fluorescent transgenic lines, we show that II-1β is transcriptionally upregulated in macrophages early during in vivo infection. Stabilization of Hif-1α upregulates II-1β transcription in macrophages in the absence of infection. II-1β signaling is required for protective NO production by neutrophils and a subsequent decrease in infection. Our data indicate that protective Hif-1α mediated NO is at least partially regulated by the key proinflammatory mediator II-1β, increasing our understanding of the mechanism of action of the potential therapeutic target, Hif-1α, as a host-derived factor in TB.

Materials and Methods
Zebrafish and bacterial strains
Zebrafish were raised and maintained on a 14:10 h light/dark cycle at 28°C, according to standard protocols (25), in U.K. Home Office–approved facilities at The Bateson Centre aquaria at the University of Sheffield. Strains used were Nacre (wild type), Tg(mpeg1:mcerry-Fliumpt3,TgBAC-ilβ:eGFP)sh445, Tg(mpeg1:mcerryCAAX)sh378, Tg(phd3:EGFP)i144, and Tg(lyz:DS-RED2)zc50 (26–30).

M. marinum infection experiments were performed using M. marinum M (no. BAA-535; ATCC) containing a pST3-mCherry or pST3 mCrinm vector (31). Injection inoculum was prepared from an overnight liquid culture in the log-phase of growth suspended in 2% polyvinylpyrrolidone (PVP) 40 solution (Calbiochem) as previously described (32). One hundred to one hundred and fifty CFU were injected into the caudal vein at 28–30 h postfertilization (hpf) as previously described (32).

Generation of TgBAC-ilβ:GFP)sh445 transgenic and II-1βH1446/Il-1βH1446 mutant zebrafish
An eGFP SV40 polyadenylation cassette was inserted at the II-1β ATG start site of the zebrafish bacterial artificial chromosome (BAC) CH-211-147h23 using established protocols (34). Inverted Tol2 elements were inserted into the chloramphenicol acetyltransferase coding sequence, and the resulting modified BAC was used to generate TgBAC-ilβ:eGFP)sh445. II-1βH1446/Il-1βH1446 mutant embryos were generated by CRISPR-Cas9 mediated mutagenesis targeted around an MwoI restriction site in the third exon of the II-1β using the method described by Hruscha et al. (35) and the template sequence 5’-AAAGCAACACCAGTGGCTCCATTITTCATGTTG-3’ (ACACTGAAACACGGAGGCAAG-3’ (Hif-1β target sequence in bold). PCR with il-1βF 5’-TAAGGAAAACTCATCTAC-3’ and il-1βR 5’-ATACGTCGACCATCTGAA-3’ and subsequent MwoI digestion were used for genotyping.

Morpholino knockdown of II-1β
The il-1β morpholino (Gene Tools) was used as previously reported (36). A standard control morpholino (Gene Tools) was used as a negative control. RT-PCR of il-1β was performed on embryos at 2 and 5 d postfertilization (dpf), as previously described (36). The following primers were used: il-1β, accession number NM_212844 (https://www.ncbi.nlm.nih.gov/nucleotide/NM_212844), forward primer: 5’-ATGGCATGCGGGCAATATGAA-3’, reverse primer: 5’-CAGTTCACGGTTGGGACATGAA-3’, forward primer: 5’-ACACTGAAACAGGAGCAAG-3’, reverse primer: 5’-CAATCCGAACATCTTCCCCGAC-3’.

Confocal microscopy of transgenic larvae
1 and 4 dpi, transgenic zebrafish larvae infected with fluorescent M. marinum strains were mounted in 0.8–1% low melting point agarose (Sigma-Aldrich) and imaged on a Leica TCS SPE confocal on an inverted Leica DMi8 base and imaged using 20× or 40× objective lenses.

For quantification purposes, acquisition settings and area of imaging (in the caudal vein region) were kept the same across groups. Corrected total cell fluorescence was calculated for each immune-stained cell using Image J as previously described (18, 21).

Tailfin transaction
Inflammation was induced in zebrafish embryos by tail transection at 2 or 3 dpf as described previously (34). Embryos were anesthetized by immersion in 0.168 mg/ml Tricaine (Sigma-Aldrich), and tail transection was performed using a microscapel (World Precision Instruments).

Quantitative PCR of il-1β SYBR Green quantitative PCR (qPCR) was performed on 1dpf M. marinum infected (or PVP control) embryos as previously described (37). The following primers were used: il-1β, accession number NM_212844 (https://www.ncbi.nlm.nih.gov/nucleotide/NM_212844), forward primer: 5’-GAACAGAATGAGACCATACAACACC-3’, reverse primer: 5’-ACCGCAGCTGAAATCCACAC-3’, reverse primer, accession number AY391451 (https://www.ncbi.nlm.nih.gov/nucleotide/AY391451), forward primer: 5’-ACACTGAAACAGGAGCAAG-3’, reverse primer: 5’-CAATCCGAAACATCTTCCCCGAC-3’.

Bacterial pixel count
M. marinum mCherry–infected zebrafish larvae were imaged at 4 dpi on an inverted Leica DMi8 with a 2.5× objective lens. Brightfield and fluorescent images were captured using a Hamamatsu OrcaV4 camera. Bacterial burden was assessed using dedicated pixel counting software as previously described (38).

RNA injections
Embryos were injected with dominant hif-1α variant RNA at the one-cell stage as previously described (20). hif-1α variants used were dominant active (DA) and dominant negative (DN) hif-1α (ZFHI: hiflab). Phenol red (PR) (Sigma-Aldrich) was used as a vehicle control.

Hydroxyrase inhibitors
Embryos were treated from 32 hpf until 2 dpf by addition to the embryo water, and DMSO was used as a negative solvent control. The pan hydroxysterase inhibitor, dimethylloxaloylglycine (DMOG; Enzo Life Sciences), was used at a 100-µM concentration by incubation in E3 embryo media as previously described (20). The selective PHD inhibitor JNJ-402041935 was used on embryos at 2 and 5 d postfertilization.

Hypoxia incubation of embryos
Embryos were incubated in 5% oxygen (with 5% carbon dioxide) in a hypoxia hood (SCI-tive UM-027; Baker Ruskinn) from 32 h postinfection for 6 or 16 h and were imaged at 2 dpf. Embryos from the same clutch kept in incubated normoxic room air were used as controls.

Anti-nitrotyrosine Ab staining
Larvae were fixed in 4% paraformaldehyde in PBS overnight at 4°C, and nitrotyrosine levels were immune labeled using a rabbit polyclonal anti-nitrotyrosine Ab (60-284; Merck Millipore) and were detected using an Alexa Fluor–conjugated secondary Ab (Invitrogen Life Technologies) as previously described (18, 21).
Statistical analysis
All data were analyzed (Prism 7.0, GraphPad Software) using unpaired, two-tailed t tests for comparisons between two groups and one-way ANOVA (with Bonferroni posttest adjustment) for other data. The p values shown are *p < 0.05, **p < 0.01, and ***p < 0.001.

Results

il-1β:GFP is upregulated in macrophages during early and later stage M. marinum infection

Early M. marinum infection in zebrafish is characterized by a period of increased proinflammatory signaling (9, 13). Levels of proinflammatory cytokines have only been previously studied at a transcriptional level in whole embryos or FACS-sorted cells rather than detecting levels in situ, over time, in an intact organism (13). We hypothesized that Il-1β is a major proinflammatory cytokine that would be upregulated by both mycobacterial infection and Hif-1α stabilization. We have previously shown upregulation of il-1β message after induction of inflammation via tail fin transection by qPCR and wholemount in situ hybridization (WISH) in the zebrafish (40). il-1β is one of the most readily detectable proinflammatory cytokines during early granuloma stages of M. marinum infection and at 1 dpi (Fig. 1A) (37). At 1 dpi, transcription is upregulated 1.7-fold measured by qPCR, compared with PVP injection controls (Fig. 1A). Macrophage expression of il-1β is greatly underrepresented when measured in this way on the whole-body level because of the small proportion of cells that contribute to the immune lineage. Therefore, to investigate il-1β expression on a cellular level in vivo, we developed a BAC-derived il-1β promoter–driven GFP line, TgBAC(il-1β:GFP)Sh445, to assess il-1β expression in real time during mycobacterial infection. We sought to examine il-1β:GFP expression in our well-established inflammation assay before investigating its expression during mycobacterial infection. WISH of il-1β and il-1β:GFP does not exhibit any immune cell expression under basal conditions (Supplemental Fig. 1A, 1B, Fig. 1B). il-1β:GFP recapitulates il-1β WISH expression in response to tail transection, with upregulation observed in cells in and around the caudal hematopoietic region, consistent with immune cell expression (Supplemental Fig. 1A, 1B) (40), although, as expected, the synthesis of GFP occurs over a longer timescale than that of il-1β mRNA detected by WISH. Neutrophils are the first cells to respond to tail fin transection with increased il-1β:GFP, with fluorescence first observed at 1 h postwounding (hpw) and still present at 6 hpw (Supplemental Fig. 1C). Having demonstrated that the il-1β:GFP is responsive to inflammation in similar cells over a similar timespan as the in situ hybridization, we sought to investigate its regulation during mycobacterial infection.

We used the TgBAC(il-1β:GFP)sh445 line to show that GFP is expressed in cells proximal to M. marinum infection sites at pregranuloma phases (1 dpi) (Fig. 1B) and in larval granulomas (4 dpi) (Fig. 1C). Many of these cells contained M. marinum and had the appearance of activated immune cells with a dynamic branched phenotype (Fig. 1B, Supplemental Video 1). The earliest timepoint at which il-1β:GFP could be detected by confocal microscopy was between 6 and 8 h postinfection, (Fig. 2A), consistent with rapid transcriptional activation of the il-1β promoter postinfection and similar to the timing of macrophage il-1β:GFP expression after tail fin transection (Fig. 2B). il-1β:GFP was predominantly upregulated in infected macrophages at 1 dpi (Fig. 2C), consistent with their containment of phagocytosed M. marinum (Fig. 1B). These data demonstrate that during early stages of infection, il-1β is transcriptionally activated in infected macrophages as part of an early proinflammatory response.
Stabilization of Hif-1α upregulates il-1β:GFP at early stages of infection

We have previously shown that stabilization of Hif-1α induces neutrophil proinflammatory NO production (18, 21). We hypothesized that this may be a part of an increased proinflammatory profile in innate immune cells; therefore, we tested whether Hif-1α is upregulating a proinflammatory program in the absence of infection using the il-1β:GFP transgenic line. DA Hif-1α significantly increased il-1β:GFP expression in the absence of M. marinum infection at 2 dpf, whereas DN Hif-1α caused no difference in il-1β:GFP expression (Fig. 3A, 3B).

To assess whether stabilization of physiological levels of Hif-1α is sufficient to induce il-1β:GFP expression in the absence of M. marinum infection, embryos were treated with the hydroxylase inhibitors DMOG and JNJ-40204193 (20, 39). Hydroxylase inhibitors stabilize endogenously produced levels of Hif-1α by blocking hydroxylation by the PHD and FIH hydroxylase enzymes. Both hydroxylase inhibitors increased il-1β:GFP in the absence of infection (Fig. 3C, 3D) to a similar extent as that observed with DA Hif-1α (Fig. 3A, 3B). Finally, to understand whether a physiological stimulus could also induce il-1β:GFP expression, zebrafish were incubated in physiological hypoxia. The lowest level of oxygen zebrafish embryos tolerate without developing abnormally is 5% oxygen (and 5% carbon dioxide) (26). To demonstrate that this level of hypoxia activated Hif-1α, two time periods were tested in the Tg(phd3:EGFP)i144 transgenic line. We have previously demonstrated that phd3 is a major downstream target of hypoxia in zebrafish embryos and that this transgenic line accurately reports Hif-1α activation (26). Incubation in 5% oxygen overnight (for 16 h) mimicked the time period used for the hydroxylase inhibitors and robustly activated phd3:GFP (Supplemental Fig. 2A, 2C); however, due to the effects of limiting oxygen on other pathways (including metabolic pathways), the zebrafish at 2 dpf were developmentally delayed (denoted by smaller eyes and less pigment). Five percent oxygen incubation for a shorter 6-h period was sufficient to activate phd3:GFP by 2 dpf without any overt developmental delay (Supplemental Fig. 2A, 2B). Both time periods of 5% oxygen incubation were sufficient to increase levels of il-1β:GFP in the absence of infection to a similar extent to both hydroxylase inhibition and DA Hif-1α (Fig. 3E, 3F).

Together, these data indicate that il-1β expression is part of a proinflammatory response to increased Hif-1α levels that could aid the host response to M. marinum challenge.

Inhibition of il-1β increases M. marinum burden and inhibits the Hif-1α NO response

IL-1β is a major proinflammatory cytokine that in many infections is instrumental in coordinating the immune response (41, 42). We sought to test whether Il-1β was important in early M. marinum infection. When functional Il-1β was blocked using a well-characterized and validated il-1β morpholino (Supplemental Fig. 3A), the morphants showed significantly increased infection compared with control morphants (Fig. 4A, 4B).

We have previously shown that stabilization of Hif-1α induces proinflammatory neutrophil NO production, via inducible NO synthase (iNOS) (18, 21). DA Hif-1α was not sufficient to reduce M. marinum infection levels when il-1β expression was blocked (Fig. 4A, 4B), suggesting that the il-1β response to M. marinum infection is critical to control infection. These results were supported by generation of an il-1β null mutant (il-1β<sup>−/−</sup>/il-1β<sup>−/−</sup>)
Supplemental Fig. 3B–E) in which DA Hif-1α also did not decrease infection, whereas in wild type siblings, infection was reduced (Fig. 4C, 4D).

NO production is found primarily in neutrophils after *M. marinum* infection in zebrafish larvae (Supplemental Fig. 4) (18, 21). We have previously demonstrated that inhibiting production of NO by Nos2 can block the antimicrobial effect of DA Hif-1α (18). Blocking Il-1β production also significantly dampened the neutrophil NO response after *M. marinum* infection at 1 dpi (Fig. 5A, 5B). As we have previously observed, DA Hif-1α upregulated NO in the absence of infection (PVP), an effect that is dampened by introduction of the bacteria (*M. marinum*) through currently unknown mechanisms, (Fig. 5C, 5D) (18). In this study, we find that il-1β morpholino blocked the increased production of nitrotyrosine by DA Hif-1α in the absence of bacteria (PVP) (Fig. 5C, 5D). These results show that Hif-1α activation of Nos2 may, at least in part, be acting through il-1β activation (Fig. 6) and hint at a much more complex regulation of proinflammatory signaling by Hif-1α than simply acting on Hif-responsive elements in the promoter of Nos2.

**Discussion**

Antimicrobial resistance is a rising problem in TB infections worldwide, and there is an urgent need to understand the regulation of host immunity by TB so that we can target host-derived factors to help tackle disease. Our data identify an early proinflammatory
response, involving macrophage il-1β expression, that is important for the onset of early disease but ultimately fails to control infection leading to granuloma formation. Using a well-established zebrafish M. marinum model of TB, we show that manipulation of Hif-1α can stimulate this proinflammatory network, aiding the host fight against infection and moving toward early clearance of infection. Specifically, we identify that Hif-1α–driven Il-1β contributes to the NO response, a response we have previously shown to be host protective (18, 21).

In this study, we took advantage of a novel transgenic zebrafish line to understand the dynamics and cell specificity of il-1β production in inflammation and mycobacterial infection, with a focus on the understudied early stages (<1dpi) of the innate immune response to TB infection. We confirmed that the il-1β:GFP expression of our line was faithful to il-1β transcription by following its expression in a well-characterized tailfin transection model of inflammation and comparison with in situ hybridization data (40, 34). Furthermore, the expression pattern of our BAC transgenic line closely matches another recently published BAC promoter–driven il-1β transgenic (43). The il-1β:GFP line also displayed some GFP signal in muscle and epithelial cells in the tail. Similar GFP expression can be seen when driven by NF-κB response elements (44) but not by WISH, suggesting that this might be off-target expression resulting from the promoter region missing some negative regulatory elements; however, it could also be specific expression that is at too low a level to be detectable by in situ hybridization. Although previous studies have shown il-1β:GFP to be upregulated in leukocytes at a tailfin transection (43), we combined the il-1β:GFP line with leucocyte-specific transgenics to show that neutrophils are the first to respond at the wound, with macrophages both migrating to and upregulating il-1β:GFP at later timepoints.

The M. tuberculosis granuloma is widely studied, both in terms of immunohistochemistry of human granulomas and in mammalian models (45–47). These studies have demonstrated that the granuloma is rich in proinflammatory cytokine production and can have necrotic centers that may be hypoxic. This proinflammatory environment has been observed in human TB, with Il-1β found to be in high levels in pleural fluid from TB patients with granulomas present (48). In our study, we observe that the proinflammatory response is present at pregranuloma stages. Lack of a proinflammatory response has been linked to poor treatment outcomes, indicating that this host response is important even in the presence of antimycobacterial agents (49). The upregulation of proinflammatory cytokines in mycobacterial infection has also been shown in the zebrafish/M. marinum larval model of TB granulomas, but previous studies have mainly relied on immunohistochemistry and/or transcriptomics data from either whole-body larvae or FACS-sorted immune cell populations (13, 27). Using live cell imaging, we found that il-1β transcription was upregulated at the granuloma formation stage; however, we also demonstrated that it is upregulated before the granuloma stage within 6–8 h postinfection. Upon infection, il-1β:GFP expression was predominantly upregulated in infected macrophages, indicating that within the first 24 h of infection, there is a macrophage proinflammatory response. Murine and human cell studies have indicated that macrophages are able to produce Il-1β a few hours after mycobacterial challenge, indicating that an early response is also present in mammalian systems, at least on a cellular level (22, 50). Our observations are in line with our previous observation of Hif-1α signaling early postinfection (detected using a phd3:GFP transgenic line), which was also observed in infected macrophages at 1 dpi (18), indicating that il-1β, alongside Hif-1α signaling, is part of an immediate proinflammatory macrophage response. This Hif-1α activation was shown to be transient, with M. marinum rapidly downregulating this in a live bacteria–dependent manner. Of note, we have previously shown that suppressing this transient early Hif-1α signal does not affect the outcome of infection, and this observation was replicated in the study, indicating that this natural, early Hif-1α stabilization is not sufficient to control infection (18). As with Hif-1α, our Il-1β data indicate that M. marinum–triggered il-1β is not sufficient to control infection.
with subsequent widespread granuloma formation at later stages; however, if primed with high \( \text{IL-1}\beta \) and NO via Hif-1\( \alpha \), the immune response is boosted, leading to lower infection and toward early infection clearance.

We have previously demonstrated that stabilization of Hif-1\( \alpha \) can aid the zebrafish host to control \( M. \text{marinum} \) infection, at least in part by priming neutrophils with increased nitrotyrosine generated by the Nos2 enzyme (18). If the Nos2 enzyme is blocked either pharmacologically or genetically, the protective effect of Hif-1\( \alpha \) stabilization is lost (18). In our study, we show that stabilization of Hif-1\( \alpha \) upregulates proinflammatory macrophage \( \text{IL-1}\beta \) expression in the absence of an infection challenge. If \( \text{IL-1}\beta \)
activity is repressed then Hif-1α induced reduction in bacterial burden is abrogated, alongside the Hif-1α–dependent increase in NO production. These data show regulation of both Nos2 and IL-1β by Hif-1α and that Hif-1α–driven NO production is partially dependent on IL-1β induction. Both human Nos2 and IL-1β have HIF-responsive elements in their promoters, and direct regulation by HIF-α signaling has been previously demonstrated in vitro (51, 52). The link between HIF-1α and IL-1β has been previously demonstrated in murine macrophages via inflammatory activation by succinate, in the absence of infection (53). In a murine model of M. tuberculosis, it was found that HIF-1α is critical for IFN-γ–dependent control of M. tuberculosis infection, but it has not previously been demonstrated that HIF-1α is important for innate defense of macrophages against M. tuberculosis (54). Our data do not rule out direct regulation of Nos2 by Hif-1α, as blocking IL-1β is likely to have wider spread immune effects; however, they do suggest that Nos2 is partially upregulated by IL-1β in the stabilized HIF-1α context. These observations, alongside our finding that blocking IL-1β, primarily observed in macrophages, can block Hif-1α–induced neutrophil nitrotyrosine production, indicate a close interplay between macrophages and neutrophils during early mycobacterial infection that is not yet fully understood.

IL-1β is an important proinflammatory component and is one of the cytokines that has been shown to be transcriptionally depressed during the 6 h to 1 dpi period of M. marinum/zebrafish pathogenesis (13). Although this depression was not detectable using the il-1β-GFP line, presumably owing to the early transcriptional response postinfection coupled with the stability of the GFP protein, our data indicate that increased il-1β transcription due to Hif-1α stabilization during this early stage of M. marinum infection is protective to the host. Alongside transcription, the processing of IL-1β by caspases plays a crucial role in immune cell pyroptosis mediated by the inflammasome (54). Recent findings in the M. marinum/zebrafish model indicate that neutrophils and macrophages can efficiently phagocytose bacteria and undergo rounds of cell death and reuptake during the initial days of infection (9). Although in this study we show a role for early proinflammatory IL-1β transcription during M. marinum infection, the role of IL-1β processing and inflammasome induced pyroptosis/cell death in these early M. marinum immune processes remain undetermined.

In conclusion, our data demonstrate an early proinflammatory response of M. marinum–infected macrophages in vivo. By stabilizing Hif-1α, macrophage IL-1β can be primed in the absence of infection and is protective upon M. marinum infection via neutrophil NO production. Therapeutic strategies targeting these signaling mechanisms could decrease the level of initial mycobacteria in patients and act to block the development of active TB by reactivation of macrophage proinflammatory stimuli. Furthermore, our findings may have important implications in other human infectious diseases in which the pathogen is able to circumvent the proinflammatory immune response to allow its survival and proliferation. Therapies that target host-derived signaling pathways such as these would be beneficial against multidrug resistant strains and could act to shorten the currently long antibiotic therapies required to clear TB from patients.

Acknowledgments

We thank the Bateson Centre Aquarium Team at the University of Sheffield for fish care. We gratefully thank Georges Lutfalla (Montpellier University) for providing the Tg(mpeg1:mCherry-FJmup2Tg) line, Lalita Ramakrishnan (University of Washington, Seattle) for M. marinum strains, and Astrid van der Sar (VU University Medical Center, Amsterdam) for the pSMT3-mCherry vector.

Thanks to Alison Condiffe and Benjamin Duffy (University of Sheffield) for use of, and invaluable help with, hypoxia hood.

Disclosures

The authors have no financial conflicts of interest.

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