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Role of eosinophils and apoptosis in PDIMs/PGLs deficient mycobacterium elimination in adult zebrafish

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ABSTRACT

The cell wall lipids phthiocerol dimycocerosates (PDIMs) and its structurally-related compound, phenolic glycolipids (PGLs) are major virulence factors of mycobacterium, as shown by the reduced growth of PDIMs/PGLs deficient mutants in various animal models. PDIMs/PGLs play active roles in modulating host immune responses. However, the cellular and molecular mechanisms of how PDIMs/PGLs deficient mutant was eliminated in vivo are still elusive. Our aim was to investigate what host immune responses have effect on mycobacterium elimination in vivo. Using microarray, we find PDIMs/PGLs modulate divergent host responses, including chemotaxis and focal adhesion's downstream pathway and apoptosis. We examine these two host responses by Diff-Quik stain, coupled with transmission electron microscopy and TUNEL stain respectively. The ultrastructure observation showed that eosinophils appeared in WT-infected zebrafish at day 1, however eosinophils arrived was delayed to day 7 in PDIMs/PGLs-deficient mutant-infected animals. More intriguingly, apoptosis was markedly increased in PDIMs/PGLs-mutant infected zebrafish at day 1 after infection, compared to WT-infected fishes at this time. However, apoptosis trend was fully reversed by day 7, with increased apoptosis were detected in WT-infected zebrafish compared with the PDIMs/PGLs-deficient mutant, especially more apoptosis within the granuloma. This study shows that the anti-apoptotic effects of PDIMs/PGLs and the recruitment of eosinophils in tissue during the early infection in zebrafish might promote bacterium growth in vivo.

1. Introduction

The control of intracellular infection with mycobacterial pathogen is dependent on both innate and adaptive immune responses. Mycobacterium have evolved in response to the host immune system and adapted to survive in host by using diverse strategies (Russell, 2007; Russell et al., 2010). One of the most impressive features of pathogenic mycobacterium is the rich lipids and complex composition of their envelope in the cell wall (Draper, 1991; Brennan and Nikaido, 1995), such as trehalose 6,6′-dimycolate (TDM), lipoarabinomannan (LAM), phthiocerol dimycocerosates (PDIMs), phenolic glycolipids (PGLs), and so on (Daffe and Draper, 1998; Draper, 1991; Onwueme et al., 2005; Brennan and Nikaido, 1995; Smith, 2003). A critical group of lipids, PDIMs and PGLs, are structurally related and non covalently bound to the outer cell wall layer, and have been studied intensively due to effect on mycobacterium virulence (Brennan, 2003; Camacho et al., 1999; Cox et al., 1999; Daffe and Draper, 1998; Brennan and Nikaido, 1995; Reed et al., 2004; Smith, 2003). Mutant strains of mycobacterium lacking surface lipids PDIMs or PGLs exhibit a strong growth defect in various animal models (Camacho et al., 1999; Cox et al., 1999).

Several studies have suggested that PDIMs/PGLs could influence the interaction of pathogenic mycobacterium with the host immune system through variety ways (Astarie-Dequeker et al., 2009; Brennan, 2003; Camacho et al., 1999; Cox et al., 1999; Sinsimer, 2008; Murry et al., 2009a,b; Reed et al., 2004; Yu et al., 2012). PDIMs has been shown to be involved in resistance to RNI. Rousseau et al. demonstrated that PDIMs contribute to the initial growth of the mycobacterium in mice by protecting it from the nitric oxide-dependent killing of activated macrophages and regulate the

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Table 1
Genes involved with cell chemotaxis and migration which were induced by infection with WT or PDIMs/PGLs deficient mutant.

UniGene_ID	Gene_Symbol	Gene_Title	Foldchange(WT/Mutant)
Dr.84258	cxcr4a	Chemokine (C-X-C motif) receptor 4a	2.042003
Dr.133987	CH211-89F7.4	chemokine CCL-C5a	0.208766
Dr.29197	LOC563952	chemokine CCL-C24 m	3.026555
Dr.117782	cxcl14	chemokine (C-X-C motif) ligand 14	3.122728
Dr.75485	cxcr4b	chemokine (C-X-C motif), receptor 4b	2.184751

production of key inflammatory cytokines such as TNF-a (Rousseau et al., 2004). Astarie-Dequeker's group showed that PDIMs mediate a receptor-dependent phagocytosis of *Mycobacterium tuberculosis* and prevent phagosomal acidification via reorganization of the plasma membrane to create a protective niche (Astarie-Dequeker et al., 2009). In addition, PDIMs could also collaborate with the secreted protein, ESAT-6 to induce cell death and cell-to-cell spread of *M. tuberculosis* (Aguiló et al., 2013). It was reported that treatment of mouse macrophages with purified PGLs inhibits the production of the pro-inflammatory cytokines TNF-α, IL-6 and CCL2 (Reed et al., 2004). The activity of PGLs on the modulation of the immune response was also supported by independent results showing that the production of this lipid in the laboratory strain H37Rv, is usually unable to synthesize it, or modify cytokine secretion by infected macrophages(Sinsimer et al., 2008). Little more was known about the biological activities of PDIMs/PGLs until recently. Cambier et al. found that PDIMs mask underlying PAMPs, and thus prevent recruitment of microbicide macrophages. On the other hand, PGLs are involved in the chemokine receptor 2 (CCR2)-dependent recruitment of permissive macrophages (Cambier et al., 2014). The work of Day TA's group showed that *M. tuberculosis* cells deficient in the complex surface lipid PDIMs are susceptible to killing by an unknown early host innate response (Day et al., 2014).

Despite progress on the function of PDIMs and PGLs has been made in recent years. Our current understanding of immune responses modulated by PDIMs/PGLs is primarily based on the use of cultured cells, primary macrophage. However, the attenuated growth of PDIMs/PGLs-deficient mutant in animal models was not observed in vitro. We and others have shown that the absence of PDIMs/PGLs did not affect the ability of mycobacterium to replicate in macrophages (Astarie-Dequeker et al., 2009; Murry et al., 2009a,b; Pethe et al., 2004; Rousseau et al., 2004; Scandurra et al., 2007). The growth of PDIMs/PGLs-deficient mutant and Wild-type strain have similar growth patterns within cultured cells, like THP-1 or RAW264.7 macrophages. Therefore, the question is whether the mechanism discovered now could account for the attenuation phenotype of the PDIMs/PGLs-deficient mutant in vivo.

M. marinum, a close genetic relative of *Mycobacterium tuberculosis* (*M. tuberculosis*), can cause typical tuberculosis in fish and other ectotherms. *M. marinum* was widely used to study the pathogenesis of *M. tb* because of some advantages (Chan et al., 2002; Gao et al., 2003; Pozos and Ramakrishnan, 2004; Stamm et al., 2003). Zebrafish, an important vertebrate model organism in scientific research, is considered as a more natural model for studying host–mycobacterium interaction due to their genetically tractable (Davis and Ramakrishnan, 2009; Parikka et al., 2012; Swaim et al., 2006). Recently, our study proved that both PDIMs and PGLs are required for the virulence of *M. marinum* in adult zebrafish infection. The growth of PDIMs/PGLs-deficient mutant in the livers of zebrafish was attenuated dramatically (Yu et al., 2012).

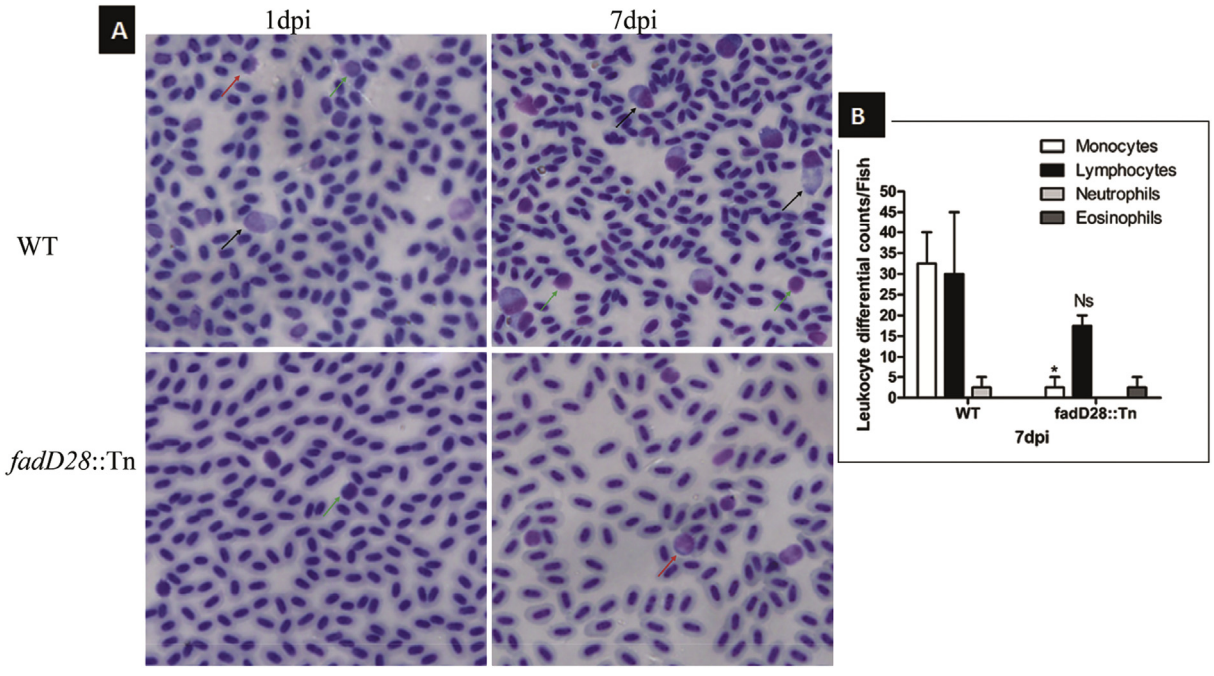

Fig. 1. Activation of adult zebrafish peripheral blood leukocytes after infection. (A) Representative photomicrographs of adult zebrafish peripheral blood leukocytes after infection with *M. marinum* at 1 and 7 dpi. Monocytes (black arrows) are recognized by their large size, abundant, foamy, basophilic cytoplasm, and irregularly-shaped nuclei. Eosinophils (red arrows) illustrate characteristic pink cytoplasm and peripheral nucleus. Lymphocytes are indicated by green arrows. Diff-Quik stain. 40 × magnification. (B) Quantitative of adult zebrafish peripheral blood leukocytes at day 7 of different groups' infection. Data point represent the mean number of leukocytes from 10 zebrafish. Counts of leukocytes at day 1 are too few to count. The "*" indicates significant difference from the WT at $p < 0.05$. Ns indicates no significant difference from the WT. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)