Subversion of host immune responses by *Mycobacterium tuberculosis*

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Abstract

Tuberculosis, one of the world's oldest diseases has nowadays reached a pandemic prevalence. Despite its long history and intense research, efficient drugs against its causative agent, *M. tuberculosis*, are still limited. One reason for the pathogen's success lies within its capability to evade host immune defense mechanisms and to create a niche within host cells enabling the bacterium to persist for long periods. *M. tuberculosis* has evolved a diversified set of strategies to manipulate the immune response of the host. In this communication, we discuss some of the strategies employed by *M. tuberculosis* in order to survive within the hostile environment of the macrophage. A detailed analysis of the molecular basis of host-pathogen interactions will unravel novel mechanisms and might contribute to finding novel approaches to treat and combat tuberculosis.

Introduction

Pathogenicity is directly related with the capacity of microbes to survive within the host. In order to circumvent destruction by host defense mechanisms, bacteria, in general, have evolved various strategies that enable the microbes to survive and replicate within host cells. These strategies utilized by pathogenic bacteria encompass prevention of lysosomal delivery, adaptation to bactericidal compartments, formation of specialized protective vacuoles and escape from the phagosome into the cytoplasm (Rosenberger and Finlay 2003; Cossart and Sansonetti 2004). Generally, pathogenic bacteria express effector proteins and/or lipids which are either structural components of the cell envelope or secreted molecules, so-called virulence factors that induce or block distinct host responses which are beneficial respectively detrimental to the pathogen.

In mycobacteria, several of these factors which interfere with the trafficking route of mycobacteria from phagosomes to lysosomes have been identified and can be classified as either being of lipid, oligosaccharide or protein origin. Through the secretion of virulence factors, mycobacteria modulate phagosome maturation, such that their degradation within the bactericidal environment of lysosomes is prevented. Several of these virulence factors are classified as enzymes which cleave or transfer phosphate groups (SapM, PtpA, PtpB, PknG), others are proteins encoded by a specific virulence locus termed RD1 (region of difference 1) or glycolipids that interfere with vesicular trafficking.

Microbial factors

One target that is manipulated by effector proteins from pathogenic mycobacteria is PI3P (Stefan S. Weber 2009), a phosphoinositide glycolipid that is crucial for phagosome maturation. Phophoinositol (PI) lipids including the phosphorylated PI3P recruit downstream effector proteins such as EEA1 (early endosomal antigen 1) and Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) (Fratti, Backer et al. 2001) that, through the hydrolysis of GTP, bind to PIs via specific motifs (FYVE, FERM, ENTH/ANTH, PH, PX) (Downes, Gray et al. 2005; Lemmon 2008). Mycobacterial SapM, a lipid phosphatase, dephosphorylates and depletes PI3P from the phagosome resulting in the arrest of phagosome lysosome biogenesis (Vergne, Chua et al. 2005). Phosphatase PtpB carries out a similar function, but has broader substrate specificity. It functions by dephosphorylating all mono-phosphorylated PI forms (Beresford, Mulhearn et al. 2009; Stefan S. Weber 2009). Moreover, lipid phosphatase PtpA dephosphorylates the host protein vacuolar protein sorting (VPS) 33B, which when inactivated, cannot generate GTP-activated Rab7, leading to an arrest of phago-lysosome biogenesis (Bach, Papavinasasundaram et al. 2008). Another secreted mycobacterial

protein with a thus far undefined target is protein kinase G (PknG), one of eleven serine/threonine protein kinases expressed in pathogenic mycobacterial species including *M.* tuberculosis (Cole 1998; Cole, Brosch et al. 1998; Av-Gay and Everett 2000). PknG is released into the cytosol of macrophages where it prevents lysosomal delivery; deletion of the *pknG* gene or inactivation of the kinase by a specific inhibitor (Walburger, Koul et al. 2004; Scherr, Honnappa et al. 2007) decreases mycobacterial survival rates significantly. It is likely that PknG phosphorylates a host molecule directly involved in phagosome maturation or in the regulation of macrophage activation. However, the exact function of PknG within the host cell remains to be established.

Apart from the secreted phosphatases and kinases, structural components of the mycobacterial cell wall, namely lipids, play a major role in PI metabolism and therefore also promote prevention of lysosomal delivery. Lipoarabinomannan (LAM), an analogue of glycosylated eukaryotic phosphatidylinositol as well as its precursor phosphatidylinositol mannoside (PIM) prevent maturation of the mycobacterial phagosomes into bactericidal phagolysosomes. LAM adopts a crucial role by inhibiting hVPS34, a calmodulin-dependent PI3K kinase that generates PI3P on the phagosomal membrane ((Vergne, Chua et al. 2003), see also below). PIM on the other hand is involved in promoting fusion between phagosome and early endosomes in a PI3K independent manner (Chua and Deretic 2004; Chua, Vergne et al. 2004; Vergne, Fratti et al. 2004).

Another major virulence determinant is represented by the mycobacterial ESX-1 system (early secretory antigenic target system 1) encoded by the RD1 (region of difference 1) genomic region. The ESX-1 system, a specialized protein secretion system, is responsible for secretion of ESAT-6 (early secretory antigenic target), CFP-10 (culture filtrate protein 10) and EspA (Gao, Guo et al. 2004; Fortune, Jaeger et al. 2005; DiGiuseppe Champion and Cox

2007). Defects in ESX-1 mediated secretion lead to increased lysosomal delivery rate and degradation of mycobacteria within lysosomes (MacGurn and Cox 2007; McLaughlin, Chon et al. 2007). However, secreted ESAT-6, CFP-10 and EspA do not seem to be responsible for mycobacterial interference with phagosomal trafficking. (Gao, Guo et al. 2004; MacGurn and Cox 2007) and therefore it is likely that other mycobacterial virulence factors, that themselves depend for their secretion on the ESX-1 secretion system, participate in the block of lysosomal delivery.

Host factors

Only a few host factors involved in mycobacterial pathogenesis have been identified and characterized to date. Unlike most phagosomes containing cargo such as beads, heat killed or non-pathogenic mycobacteria, phagosomes containing live, pathogenic mycobacteria fail to mature into phagolysosomes. Mycobacteria shape their own phagosomal niche within the otherwise hostile macrophage environment by actively retaining or eliminating a set of host proteins which is needed to allow phagosome maturation and acidification (Pieters and Gatfield 2002; Gatfield and Pieters 2003).

One host protein which is excluded from the mycobacterial phagosome is the previously mentioned phosphatidylinositol-3-kinase and its product phosphatidylinositol-3-phosphate (PI3P) (REF.). PI3P interacts with and recruits proteins containing a FYVE domain (Fab1p, YOTB, Vac1p and EEA1). Two molecules required for the fusion of early and late endosomes and are excluded from mycobacterial phagososomes as a consequence of reduced PI3P generation are the early endosomal antigen 1 (EEA1) and hepatocyte growth factor regulated tyrosin kinase substrate (Hrs) (Fratti, Backer et al. 2001; Mueller and Pieters 2006).

Another extensively studied host molecule is coronin 1 that is actively retained by live, pathogenic mycobacteria around the mycobacterial phagosomes and essential for bacterial survival (Ferrari, Langen et al. 1999).

Coronin 1, also known as TACO or P57, is a member of the WD repeat containing protein family of coronins. The first coronin molecule to be identified was *Dictyostelium* coronin, which has been implicated in the regulation of actin based cellular processes such as phagocytosis and migration. Based on the homology to the single coronin isoforms expressed in *Dictyostelium* all thus far identified seven mammalian coronin family members have been classified as F-actin regulators (de Hostos 1999; Rybakin and Clemen 2005). However, when coronin 1 deficient leukocytes were analyzed, no actin related defects were apparent. Instead of regulating F-actin dynamics, coronin 1 was found to be not only retained around mycobacterial phagosomes (Ferrari, Langen et al. 1999; Jayachandran, Sundaramurthy et al. 2007; Jayachandran, Gatfield et al. 2008; Mueller, Massner et al. 2008) but essential for mycobacteria induced elevation of cytosolic calcium levels and activation of the phosphatase calcineurin. Calcineurin phosphatase activity has been implicated in a large variety of cellular processes covering the range from transcriptional activation to dephosphorylation of proteins involved in endocytic processes and degradation of signalling molecules in order to shut down receptor mediated signalling cascades (Aramburu, Heitman et al. 2004; Pieters 2008). The phenotype of coronin 1 deficient macrophages could be mimicked by incubation of wild type cells with the calcineurin blockers cyclosporine A or FK506 which induced lysosomal delivery of the pathogen as observed in the absence of coronin 1. On the other hand, mycobacteria were prevented from being delivered to lysosomes in the absence of coronin 1 by artificially increasing cytosolic calcium levels and activating calcineurin, by use of the calcium ionophore calcimycin. These results clearly established coronin 1, calcium mediated

signalling events and calcineurin as key players during mycobacterial infections as well as pathogen survival (Pieters 2008; Pieters 2008).

Pathogenic mycobacteria do not only exclude or retain host factors from or at their phagosome but in addition directly impact the activation state of macrophages (Korbel, Schneider et al. 2008). Keeping macrophages in a non-activated state is essential for mycobacterial survival as the mechanisms exploited by pathogenic mycobacteria are almost exclusively functional in non-activated host cells. It has been shown that the inhibition of signalling pathways for cytokines such as interferon-γ or tumor necrosis factor-α dramatically enhances the susceptibility of both mouse models as well as humans to mycobacterial disease (Adams and Hamilton 1984; Flynn and Chan 2001; Pieters 2008). Most of the immune mechanisms activated by these cytokines such as the generation of reactive oxygen and nitrogen species as well as cell death and phagosome-lysosome fusion are still poorly understood. Recent work has implicated LRG-47, a member of the interferon-γ regulated family of p47 GTPases in restricting the intracellular growth and survival of pathogenic mycobacteria. In uninfected cells LRG-47 is associated mainly with Golgi membranes but becomes associated with the plasma membrane and is co-phagocytosed upon mycobacterial entry. Consistent with these findings mice lacking LRG-47 are highly susceptible to mycobacterial infections and are unable to control growth of the pathogen. This effect may at least in part be explained by the finding that mycobacterial phagosomes carry lower levels of v-ATPase in the absence of LRG-47, which is needed for the acidification and consequently maturation of phagososomes to phagolysosomes (MacMicking, Taylor et al. 2003; Feng, Collazo-Custodio et al. 2004).

Concluding remarks

The identification and characterization of additional mycobacterial as well as host cell factors involved in this host-pathogen interplay will not only allow for a better understanding of the virulence mechanisms exploited by pathogens such as *Mycobacterium tuberculosis*, but also shed new light on the mechanisms guiding the co-evolution of pathogens with their host cells. Detailed knowledge of the molecular mechanisms of host-pathogen interaction will contribute towards the development of new strategies for fighting microbial diseases, the identification of novel drug targets as well as the development of more efficient treatments.

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