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Response of Mycobacterial Species to an Acidic Environment

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1. Introduction

Bacteria must be able to respond to a host of environmental stresses. The ability is vital if bacteria are to withstand insults both of an external location as well as during infection of an animal host. Many investigators study these responses individually in order to better understand pathogenic processes. Desiccation, response to UV light, heat, and cold stress can be encountered in the external environment. Low oxygen tension, heat stress, oxidative stress, nitrosidative stress, and acidic stress are all environments that can be encountered upon infection *in vivo* of a host. This review focuses on acidity as a stress which can be important in mycobacterial pathogenesis. Acidity can be found in such environments as acidic water and soil and can affect mycobacteria in an animal host. Mycobacteria encounter acidic stress at sites of inflammation and within phagosomes of macrophages. Exposure to acidic stress in the external environment may prime mycobacteria to upregulate genes involved in pathogenesis. Upregulation in response to acidic stress may prime mycobacteria to be more resistant to other stresses and to be more able to persist *in vivo*.

2. Environmental mycobacteria

In contrast to *Mycobacterium tuberculosis* which is found exclusively in a human or animal host, environmental mycobacteria are found in the environment. Contamination of water or other environmental locations can then lead to infection. Environmental mycobacteria are in general not transmitted person to person but instead environment to person. Therefore the environmental milieu is important in priming mycobacteria for survival. If acidity is encountered in the host upon infection of a human, those genes that aid in resistance in the environment are already upregulated. Upon being engulfed by macrophages acid primed mycobacteria are already somewhat resistant to that *in vivo* environment.

Mycobacterium avium has been found to be isolated from acidic swamp waters in the Southeastern United States (Kirschner et al; 1992). *M. avium* isolates grew at pH levels as low as 4.0 and grew at acidic pHs just as well as at neutral pH (Kirschner et al; 1999). Mycobacteria have also been isolated from brook waters draining from acidic coniferous forests in Finland. Furthermore, growth seems to be negatively correlated with pH. As pH lowers there is a propensity to isolate mycobacteria (Livanainen et al; 1999). Thus low pH

may favor isolation of mycobacteria from environmental samples. In fact *Mycobacterium parascrofulaceum* has been isolated from acidic hot springs with a pH as low as 3.0 in Yellowstone National Park. (Santos et al; 2007). In addition *Mycobacterium montefiorense* was isolated from soils at pH 2.0 (Uyttebroek et al; 2007). It seems that mycobacteria that grow at neutral and low pH behave differently from other environmental bacteria that grow at neutral pH but poorly at acidic pH. Thus environmental mycobacteria seem to have exploited an environmental niche that other bacteria have a difficult time occupying. Mycobacteria such as *Mycobacterium avium* as well as others are common contaminants of municipal water supplies where they serve as a reservoir to infect human beings. In fact in areas where there is a prevalence of mycobacteria in acidic waters, there also is an increased prevalence of infections due to these environmental mycobacteria. It is tempting to speculate that environmental mycobacteria present in acidic conditions are more resistant to acidic environments in a human or animal host. In addition those mycobacteria which are exclusively human pathogens may have evolved from ancestral mycobacteria that already demonstrated an exquisite ability to withstand acidic stress. Thus it is no wonder that mycobacteria within the *Mycobacterium tuberculosis* complex have evolved to occupy and resist the partially acidic environment of the phagosome of alveolar macrophages.

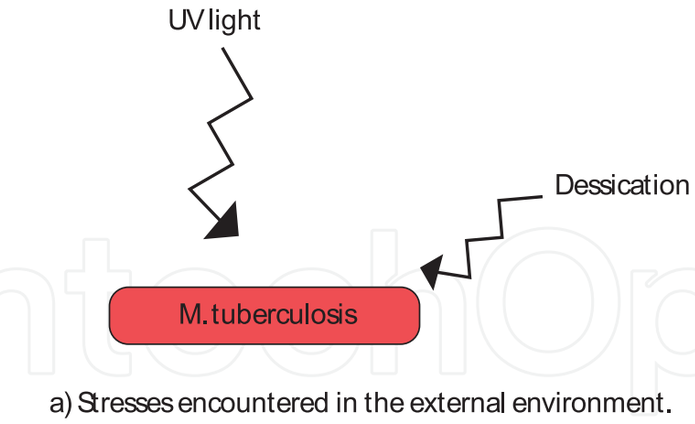
Mycobacteria may bear an intrinsic ability to resist environmental stresses. The cell wall and outer membrane help to protect environmental mycobacteria from acidic stress. Mycobacteria contain a thick cell wall surrounding an inner membrane. The cell wall is partially composed of a peptidoglycan-arabinogalactan polymer and is quite thick. In addition they contain an outer membrane that is somewhat different from what is found in gram negative organisms. The mycobacterial outer membrane is composed of mycolic acids in addition to other components and the lipid structure may serve to protect mycobacteria from environmental stresses (Hoffman et al; 2008, Neiderweis et al; 2010). It has been proposed that the mycobacterial cell wall and outer membrane rich in lipids act as an effective barrier against the entry of protons (Mechnikoff, 1905). In support of the idea that the waxy lipid rich cell envelope inhibits the toxic effects of acidic stress, many acid susceptible mutants are within genes that are predicted to have cell wall functions or are involved in lipid metabolism (Fisher et al; 2002, Saviola et al; 2002, Vandal et al; 2009).

While mycobacteria may have an intrinsic ability to resist acidic stress, they also bear inducible responses to acidic stress. One of these inducible systems is the acid tolerance system (ATR) where prior exposure to acidic stress confers protection upon subsequent exposure with more extreme acidic stress. Mycobacteria possess a blunted acid tolerance system as it confers only 2-3 fold protection if the mycobacteria are first exposed to pH 5.0 and then challenged with pH 3.0 (O'Brien et al; 1996). This is much lower than what is found in enteric bacteria that exhibit a 1,000 to 10,000 fold protection against acidic stress if they are conditioned prior with a mild acidity. Thus while mycobacteria possess an acid tolerance response it is much less protective than in gram negative organisms.

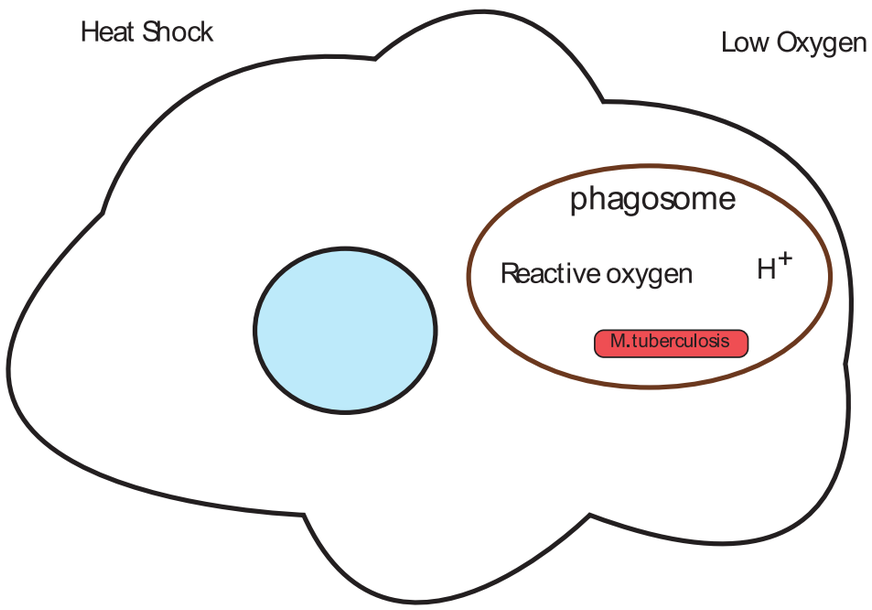
Environmental mycobacteria respond to acidic stress by upregulating a variety of genes presumably necessary to resist this stress. In addition there are examples of a number of genes that when mutated result in mycobacteria that are more sensitive to acidic stress. Investigations into the stressome of *Mycobacterium avium subsp. paratuberculosis* revealed that 195 genes are upregulated at acidic pH (Wu et al; 2007). *M. avium subsp. paratuberculosis* is the causative agent of Johne's disease and has been implicated in

Crohn's disease in humans. This mycobacterium is exposed to acidic pH in the alimentary tract of cattle and is also exposed to acidity within the phagosomes of macrophages of which it is an intracellular parasite. In addition *M. avium subsp. paratuberculosis* may encounter acidity within sites of inflammation. The large number of genes in *M. avium subsp. paratuberculosis* that are upregulated in response to acidic stress indicates that this is an important environmental condition *in vivo*. Of the 195 genes upregulated by acidic stress, 6 of the genes are common between heat shock and acidic stress. In addition general stress response genes *htpX*, *clpX*, and *relA* were upregulated. This implies that there is a common stress response pathway that includes both acidity and heat shock. Genes involved in mycolic acid synthesis were upregulated at acidic pH, as well as mycobactin metabolism and a protein kinase (*pknB*). Genes involved in mycobacterial cell entry (*mce1* and *mce4*) and a transcriptional regulator *kpnE* were also upregulated. Many genes were also downregulated and these outnumbered the genes which were upregulated by acidic stress. Perhaps acidic pH is a toxic environment that serves to down regulate transcription in general. This makes those genes that are upregulated functionally even greater in their responsiveness. Deletion mutants in genes *aceAB*, *mbtH2* and *prrA* that were positively regulated by acidic stress were evaluated for growth in mice. Consistent with these genes being important in pathogenesis, infection with these strains resulted in a 1-2 log lower level of colonization of the liver compared to wild type *M. avium subsp. paratuberculosis*.

Mycobacterium smegmatis is a bacterium originally isolated from human smegma (Rose et al; 2009). It has been the cause of opportunistic infections and catheter related infections (Brown et al; 1999, Newton et al; 1993). *M. smegmatis* may encounter acidity at various locations on the surface of the human body including the skin. If this bacterium is successful in invading a human host, it will invariably be engulfed by macrophages where it will encounter acidity within the phagosomes of macrophages. In addition phagosomes of macrophages which contain *M. smegmatis* may have a lower pH than phagosomes of macrophages that have more virulent *M. tuberculosis*. This is presumably because *M. smegmatis* does not inhibit phagosome maturation to the same level that *M. tuberculosis* does. Studying proteomic responses during acidic stress in *M. smegmatis*, 52 proteins were found to be increased in abundance (Roxas and Li, 2009). Some of these proteins were predicted to be transmembrane proteins, involved in transporter activity, or fatty acid metabolism. Fatty acid metabolism could increase cell wall thickness or increase energy storage. *Acr*, encoding a protein typically associated with low oxygen tension was also induced in *M. smegmatis* as was *devSR* encoding a two component system also thought to respond to low oxygen tension. Mutants of *M. smegmatis* that could not grow in the presence of a protonophore at acidic pH were also identified using transposon mutagenesis (Tran et al; 2005). Genes disrupted include those predicted to be involved in phosphonate phosphite assimilation, lipid biogenesis, and methionine biosynthesis. A mutant in the homologue of the *M. tuberculosis lipF* gene was identified which may be involved in cell wall synthesis or energy homeostasis. Extracytoplasmic sigma factors SigE and SigF (Wu et al; 1997, Gebhard et al; 2008) are required by *M. smegmatis* for survival during acidic stress, indicating a global response to acidic stress. In addition a porin gene, *mspA*, is down regulated at acidic pH. Perhaps this may limit the influx of protons which may be toxic to the cell (Hillman et al; 2007).



a) Stresses encountered in the external environment.



b) Stresses encountered in a host macrophage or externally in the host body.

Fig. 1. Stresses encountered by *M. tuberculosis*. One of these stresses is acidity encountered in the human macrophage.

3. Acidic environments *in vivo* encountered by *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is exposed to a variety of acidic conditions within the human body. These can include the classical location within the phagosome of an alveolar macrophage. It may also include the centers of caseating granulomas which can be quite acidic as has been found to be true in a rabbit model of tuberculosis (Dannenberg, 2006). *M. tuberculosis* is expelled from an individual with active tuberculosis as droplet nuclei to be inhaled by a contact. Once inside the lungs of the newly infected individual the bacilli are engulphed by alveolar macrophages and encounter the internal environment of the phagosome. When macrophages phagocytose latex beads or less virulent bacteria the

phagosomes mature, lower their internal pH to as much as 4.5, and fuse with lysosomes to form a phagolysosome. *M. tuberculosis* however appears to modulate the phagosome and inhibit the maturation process (Armstrong and Hart, 1971, Sturgill-Koszycki et al; 1994, Huynh and Grinstein, 2007). This seems to occur partly by exclusion of the proton ATPase from phagosomes containing *M. tuberculosis*. *M. tuberculosis* also seems to freeze the phagosome prior to fusion with the lysosome. The internal pH lowers to approximately 5.5 and can rebound to pH 6.5. Activation of macrophages by INF-γ results in activation of the macrophage and seems to overcome the block in phagosomal maturation. Phagosomes containing *M. tuberculosis* within macrophages stimulated with INF-γ can reduce their pH to 4.5 or lower (Schaible et al;1998, Via et al, 1998,MacMicking et al; 2003, Ehrt and Schnappinger, 2009). For this reason immune status is so important in determining susceptibility in developing an *M. tuberculosis* active infection. Thus the immune response to *M. tuberculosis* can be imperative in controlling replication of the bacilli. Likewise the ability to survive and thrive within the phagosome of an activated macrophage is vitally dependent on the bacilli's ability to respond to acidic stress. It is interesting to note that interfering with the drop in acidity abrogates the upregulation of approximately 80% of the genes which are normally upregulated in *M. tuberculosis* within the macrophage (Rohde et al; 2007). This is more evidence that acidity is one of the key environmental stresses and signals that *M. tuberculosis* encounters *in vivo*.

4. *Mycobacterium tuberculosis* responses to acidic stress

M. tuberculosis responds to the acidic environment in a number of ways. While *M. tuberculosis* may be able to grow at a slightly acidic pH it does not seem to replicate below pH 5.5(Chapman and Bernard, 1962; Portales and Pattyn, 1982). It is unknown if *M. tuberculosis* has an acid tolerance system as is found in *M. smegmatis* (O'Brien et al; 1996). The cell wall and outer membrane are likely to provide a robust intrinsic resistance. In addition a number of genes have been found to be upregulated by acidic stress. This constitutes an adaptive response to the environment that may be very necessary for survival. In addition some genes have been identified that when mutated result in dramatic acid sensitivity, however those genes are expressed constitutively.

pH may be the main signal to which mycobacterial pathogens respond. By interfering with the development of acidity within phagosomes with the addition of concanamycin A, researchers could assess the effect on mycobacterial transcription. Treatment with concanamycin A abrogates much of the transcriptional response. Prevention of the normal drop in pH to 6.4 and maintaining neutral phagosomal pH resulted in 80% of the genes normally upregulated in macrophages failing to be upregulated (Rohde et al; 2007). Thus this points to the importance of acidity in defining the phagosomal environment.

The promoter of the *lipF* gene is known to be upregulated by acidic stress at pH 6.4 to as low as pH 3.6 (Richter and Saviola, 2009). This promoter region from *M. tuberculosis* is uppregulated in fully virulent *M. tuberculosis* as well as in the environmental mycobacterium *M. smegmatis* (Saviola et al; 2002). Thus this shows that in at least this particular case environmental mycobacteria and *M. tuberculosis* complex mycobacteria can respond in a similar way to acidity. Interruption of the DNA region between the *lipF* promoter and the gene resulted in a bacterium much more attenuated for growth in mouse lungs and macrophages indicating *lipF* is important in pathogenesis (Camacho et al; 1999).

M. tuberculosis genes upregulated by acidic conditions found in phagosomes were analysed by microarray analysis and revealed a number of genes which were upregulated at 15 and 30 minutes. These include the *mymA* operon which appears to be under the control of VirS an AraC/XylS family transcription factor (Fisher et al; 2002). When the *mymA* operon was deleted it was revealed to be important for cell wall ultrastructure. The operon was also important for persistence in the spleens of guinea pigs (Singh; 2005). In separate studies the global regulators SigH and SigB were induced by acidic stress implying that these sigma factors are important in responding to acidity (Rohde et al; 2007). *AprABC* is also upregulated at acidic pH and encodes a likely RNA or DNA binding protein. *AprAB*C is expressed in macrophages at acidic pH and is dependent on the two component system *phoP/R* (Abramovitch et al; 2011). Another gene *ompATb* was induced at pH 5.5 and a mutant was attenuated in mice for growth (Raynaud et al; 2002). *OmpATb* encodes a porin that functions specifically at low pH and has been shown to export ammonia. Therefore in the closed environment of the phagosome this porin presumably senses acidic stress and responds by pumping ammonia into the environment in order to lower and neutralize the surrounding acidity (Song et al; 2011). The two component system Rv0902c and Rv903c are thought to regulate *ompATb*.

A transposon mutagenesis study was done to identify *M. tuberculosis* mutants sensitive to acidic pH (Vandal et al; 2008, Vandal et al; 2009). 21 genes were identified of which 15 were shown to be involved in cell wall functions bolstering the idea that the cell envelope is important in protection from acidic stress. When the 21 genes were rescreened it was revealed that only 2 retained acid sensitivity in phosphate citrate buffer. Possibly the tween-80 added to disperse the mycobacteria and to discourage clumping had hydrolyzed into oleic acid. Fatty acids can be toxic to mycobacteria and other bacteria especially at acidic pH and the 19 genes with transposon insertions that are sensitive to acidity in the presence of tween-80 may confer resistance to this manner of stress. Two genes that retained acid sensitivity in phosphate citrate buffer were *Rv2136c* involved in peptidoglycan synthesis and *Rv3671c* a serine protease that may modify the mycobacterial envelope (Biswass et al; 2010). When these two genes were disrupted by transposon mutagenesis, the mycobacteria were unable to maintain their intracellular pH. In addition the *Rv2136c* transposon mutant was hypersusceptible to cell wall stresses such as exposure to sodium dodecyl sulphate (SDS) (Vandal et al; 2009). This is similar to what is seen with a mutation in the *mymA* operon (Singh et al; 2005). Interestingly *Rv3671c* is constitutively expressed showing that stress inducibity may not be the best indicator that a gene is necessary to withstand the stress. A mutation in the *M. tuberculosis* gene *mgtC* caused attenuation in macrophages. Impaired in growth in lungs and spleens of mice, this gene seems to be important for pathogenesis *in vivo*. Additionally, *mgtC* mutants could not grow *in vitro* in low magnesium and mildly acidic pH. A mutant in *Salmonella mgtC* had a similar phenotype. These data indicate that phagosomes are a location bearing low pH and low Mg2+ concentrations (Buchmeir et al; 2000).

Nitrate respiration seems to protect hypoxic *M. tuberculosis* against acid stress. Microaerophilic and hypoxic mycobacteria are more sensitive to acid stress. Exogenouus nitrate appeared to protect under these conditions as it seemed to serve as a terminal electron acceptor (Tan et al; 2010).

5. PhoP/PhoR and acidity

Two component systems are present in many bacterial species. These systems are composed of a sensor kinase that is capable of transmitting a signal from outside the bacterial cell to the internal regions of the cell. The signal may be acidity, low oxygen tension, or some other signal. Once the sensor kinase responds to the signal it then phosphorylates its target the transcriptional regulator, which can activate or repress transcription. Thus these two component systems are capable of sensing an external stimulus and converting it into differential gene regulation. Many genes may lie downstream of the response regulator, thus constituting a manner to coordinate the regulation of many genes to affect the appropriate response to environmental challenges.

In *M. tuberculosis* there are many two component systems. PhoP/R is one of these systems that has been analyzed extensively for its impact on the pathogenesis of this microorganism and is annotated to be involved in phosphate metabolism because of its similarity to a homologue in *Bacillus subtilus*, though it is unlikely to have this predicted function (Perez et al; 2001). PhoP is a response regulator and a member of the OmpR family of transcriptional regulators. PhoP phosphorylation modulates DNA binding by increasing its binding affinity and this occurs via protein protein contacts between phoP protein monomers (Sinha et al; 2008). However these studies were executed with *phoP* cloned and overexpressed from *M. tuberculosis* H37Ra an attenuated strain of the tubercle bacilli. In a different study PhoP from fully virulent *M. tuberculosis* H37Rv but not H37Ra was capable of binding to its own promoter (Chesne-Seck et; 2008). Mutation within the DNA binding domain of PhoP from *M. tuberculosis* H37Ra resulted in diminished DNA binding. PhoP recognizes direct repeat sequences of 9 base pairs in length and can act to repress its own promoter region (Gupta et al; 2006). This is consistent with what is found with other transcriptional regulators in other species. These response regulators are usually present at a low basal level to avoid spurious binding to non-canonical sites that may occur at higher concentrations. Once a critical level of transcriptional regulator protein is available to bind to its DNA binding site it may repress its own synthesis. When transcriptional regulator levels drop due to protein turn over, the repressor site becomes unoccupied, and more transcriptional regulator is synthesized. This is what occurs with the *Escherichia coli* arabinose responsive repressor/activator AraC (Saviola et al; 1998, Lobell and Schleif, 1990), thus ensuring a constant level of transcriptional regulator present within the bacterial cells ready to respond to external stimuli. It seems logical that PhoP would act as a repressor of itself.

The attenuated strain *M. tuberculosis* H37Ra was developed at the turn of the 20th century from a clinical strain *M. tuberculosis* H37 by passaging serially on artificial laboratory media (Steenken et al; 1934). In addition to being attenuated it was revealed that *M. tuberculosis* H37Ra contains many changes within its genome in comparison to its fully virulent counterpart *M. tuberculosis* H37Rv. Looking at the transcriptome many more genes were disregulated than would have been predicted by just looking at polymorphisms in the genome indicating that global transcriptional regulators have been affected. If PhoP as part of a two component system is disregulated this has the ability to affect many 10s or 100s of genes. Indeed *phoP* is mutated within *M. tuberculosis* H37Ra within the DNA binding domain encoding region of the gene replacing a serine with a leucine (Chesne-Seck et al; 2008).

In support of the idea that the *phoP* mutation in *M. tuberculosis* H37Ra is responsible for many of the transcriptional changes compared to *M. tuberculosis* H37Rv, comparison of *M. tuberculosis* H37Ra with a *phoP* deletion strain of fully virulent *M. tuberculosis* H37Rv showed that many genes downregulated in one were also downregulated in the other (Lee et al; 2008). Disruption of *pho*P from virulent *M. tuberculosis* results in a mutant bacterium that cannot grow in macrophages or mice and cannot grow at low Mg^{2+} (Walters et al; 2006). Complementation of *M. tuberculosis* H37Ra with *phoP* from the virulent *M. tuberculosis* H37Rv partially restored the ability of the attenuated strain to replicate in macrophages. Growth restriction of *M. tuberculosis* deleted in *phoP* can be partially overcome by adding Mg^{2+} back into the test system. It is possible that PhoP/R mutations result in cell envelope defects which as a consequence require Mg^{2+} to overcome this deficiency. In fact PhoP positively regulates *pks* 2,3,4 and *mls3* that encode enzymes for production of sulphatides, diacyltrehalose, and polyacyltrehalose which can be found in the cell envelope (Walters et al; 2006). When *pks* 2, 3, and 4 were disrupted in *M. tuberculosis* H37Rv no dramatic effect was seen in pathogenesis of the microorganism. Thus pathogenesis likely lies in other loci controlled by PhoP.

PhoP controls the expression of a variety of genes involved in lipid metabolism, respiration, initial hypoxic response, enduring hypoxic response, response to stress, and genes within the RD1 genomic region known to be deleted in *M. bovis* BCG (Gonzalo-Asensio et al; 2008). Interestingly many of the genes known to be controlled by PhoP are also upregulated by acidic stress. These genes can be upregulated by acidic pH at 6.5 or 5.5, pHs likely encountered in the phagosome of macrophages. Many of these genes under the PhoP regulon and responsive to acidic stress are within the category of controlling lipid metabolism including *pks2*, *pks3*, and *pks4* (Gonzalo-Asensio et al; 2008, Rohde et al; 2007) . *LipF* is one of the genes whose promoter had previously been shown to be upregulated by acidic stress (Saviola et al; 2001). Other genes regulated by PhoP and acidic stress such as *nark1* are involved in respiration while *Rv2390c* is involved in the enduring hypoxic response. *WhiB6* is a transcription factor upregulated by acidic stress and is controlled by PhoP (Rohde et al; 2007). In addition, PhoP controls many of the genes that are expressed upon interaction with macrophages similar to what is found in *Salmonella* PhoP (Perez et al; 2001). It is intriguing to think that PhoP not only regulates genes needed for virulence, but is also sensing acidic stress as one of its signals via the sensor kinase PhoR.

While some *Mycobacterium bovis* Bacille Calmette Guerin (BCG) vaccine strains are mutated in *phoR*, they are generally thought to be missing the RD1 region of their genome compared to fully virulent *M. bovis*. The RD1 region contains some key genes that are required for virulence including *esat-6* and *cfp-10* (Behr et al; 1999, Pym et al; 2002). This indicates that the RD1 region is important in pathogenesis; *M. tuberculosis* H37Ra, however contains the intact RD1 region (Mostowy et al; 2004). PhoP is known to control *esat-6* secretion which is a major antigen recognized by T-cells (Frigui et al; 2008). *M. tuberculosis* H37Ra mutated in *phoP* and reconstituted with *phoP* from H37Rv restablished *esat-6* secretion and recognition of this antigen by T-cells in an infected animal host indicating that Esat-6 secretion lies downstream of PhoP control (Frigui et al; 2008).

The ESX-1 system controls the secretion of a variety of proteins and this secretion system has been designated type VII secretion system (Abdallah et al; 2007). The secreted proteins are the 6 kDa early secreted antigenic target (Esat-6) and the 10 kDa culture filtrate protein

CFP-10. These two proteins form a heterodimer. Interestingly Esat-6 dissociates from CFP-10 at acidic pH and is capable of lysing membranes. *M. tuberculosis* has been observed extraphagosomally in the cytoplasm of macrophages. Mutations in *esx-1* gene result in *M. tuberculosis* bacilli that do not escape from the phagosome into the cytoplasm. This implies that Esat-6 is involved in a response to acidity (Simeone et al; 2009). However PhoP may not directly control secretion of Esat-6 via Esx-1. A secreted transcription factor EspR seems to controls the transcriptional upregulation of Rv3616c which enhances the activity of the secretory Esx-1 apparatus. (Raghavan et al; 2008). When secretory apparatus activity is high, EspR is exported from the mycobacterial cell, leading to decreased transcriptional activity and decreased secretion by Esx-1. Thus this is a feedback loop for this type VII secretion system and PhoP seems not to regulate EspR itself.

PhoP mutants have been analyzed for their efficacy as vaccine strains in animal models. A *phoP* mutation in *M. tuberculosis* resulted in more attenuation compared to *M. bovis* BCG in mice and guinea pigs. In addition the *phoP* mutant induced increased immunity and a *phoP* mutant was superior to *M. bovis* BCG as a vaccine in guinea pig model (Martin et al; 2006, Aguilar et al; 2006). Disrupted *phoP* mutant were attenuated in a Balb-C mouse model of progressive pulmonary TB in immunocompetant mice. There were few small granulomas and no pneumonic lesions. The mutant stimulated longer lasting cellular immunity (Aguilar et al; 2006). It was also attenuated in SCID mice and more attenuated than *M. bovis* BCG at a 10X higher infectious dose (Martin et al; 2006). Therefore immunocompetant patients may be better able to use a *phoP* mutant as a vaccine strain than *M. bovis* BCG which can cause disease in susceptible individuals. This is especially important in sub-Saharan Africa where the Human Immunodeficiency Virus (HIV) epidemic has created an increased number of tuberculosis cases and where much of the population is routinely vaccinated to prevent occurrence of neonatal meningitis. A *M. tuberculosis* strain deficient in *phoP* and less virulent than many BCG strains, may be adept at preventing neonatal meningitis but less likely to cause overt disease in the immunocopromised.

6. Timing of responses to acidity

There seems to be a difference in the timing of upregulation of genes involved in response to acidic stress. At 15-30 minutes a number of genes are upregulated and this seems to constitute an immediate response. This early response may be controlled by VirsS at least for the *mymA* operon as previously described (Singh et al; 2005). The *lipF* promoter part of the PhoP regulon, failed to be identified as upregulated by acidic stress at 15-30 minutes (Fisher et al; 2002). However, this promoter is upregulated after 1.5 hour, with maximum regulation occurring after 24 hours (Saviola et al; 2002). At 2 hours 24 of 44 known PhoP genes are upregulated in macrophages (Rohde et al; 2007). An even later response occurs that results in the accumulation of triacylglycerol that *M. tuberculosis* may need for long term survival under stress. Tgs1 is implicated in triacylglycerol storage and when this gene is deleted the mutant fails to accumulate triacylglycerol within mycobacterial cells. Tgs1 was not upregulated at 15 minutes by acidic shock (Fisher et al; 2002). It was however upregulated upon extended acidic conditions of 3 weeks duration (Sirakova et al; 2006, Low et al; 2009, Deb et al; 2009). Thus there are varying time frames for the response to acidic conditions. There may not be 3 discrete times, but a multitude of response times. Distinct time frames of response to acidity may correlate with transcriptional regulators which

control acid responsive genes. The kinetics of the response will be different for each regulator and as a consequence depend on the exact nature of the signal that the regulator senses. Signals may be a direct or indirect consequence of acidic damage. These questions will be important when considering acid responsive genes as drug targets. Inhibition of a gene expressed early may block establishment of infection but be ineffective during chronic infection. Likewise a drug against a gene upregulated at 3 weeks may be effective against a chronic infection but be unable to prevent establishment of infection.

7. Conclusions

Acidity is a condition that is encountered by both environmental mycobacteria and mycobacteria of the *Mycobacterium tuberculosis* complex. Mycobacteria are hypothesized to possess a large degree of innate resistance to acidic stress as well as an incredible ability to make adaptive changes to withstand acidic environments. This is certainly true for environmental mycobacteria which can survive in extreme conditions. It is interesting to speculate that pathogenic mycobacteria have evolved from environmental mycobacteria and maintain some intrinsic acid resistance as well as a rich and varied ability to respond adaptively to acidic stress. These pathogenic mycobacteria for the most part will never encounter the external environment, yet they are well suited to withstand the hostile environment within the phagosome of macrophages as well as the acidic centers of caseating granulomas. Drug targets against genes necessary to survive acidic stress may be developed to eliminate *M. tuberculosis* present within acidic environments including conditions that favor dormancy.

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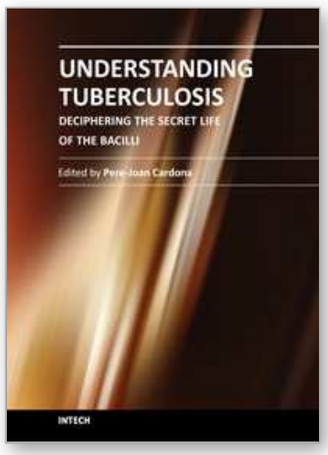

Understanding Tuberculosis - Deciphering the Secret Life of the Bacilli

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Mycobacterium tuberculosis, as recent investigations demonstrate, has a complex signaling expression, which allows its close interaction with the environment and one of its most renowned properties: the ability to persist for long periods of time under a non-replicative status. Although this skill is well characterized in other bacteria, the intrinsically very slow growth rate of Mycobium tuberculosis, together with a very thick and complex cell wall, makes this pathogen specially adapted to the stress that could be generated by the host against them. In this book, different aspects of these properties are displayed by specialists in the field.

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