

Review

Mycobacterium tuberculosis gene expression in macrophages

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Abstract

This review provides a discussion on the current information about the response of *Mycobacterium tuberculosis* to the environment encountered in the macrophage. We focus on the types of genes shown to be upregulated when the pathogen grows in macrophages and discuss the possible roles of these genes in adaptation to the conditions in the eukaryotic cell, in the context of enhancing the survival of the pathogen during infection.

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1. Introduction

The bacterial pathogen *Mycobacterium tuberculosis* generally infects its mammalian host through the aerosol route. It quickly encounters alveolar macrophages and establishes residence within those phagocytic cells. Contrary to their usual function, the alveolar macrophages do not eliminate the bacteria, but instead they provide a supportive environment for the replication of their unwelcome guests. Therefore, one of the keys to an understanding of the pathogenicity of *M. tuberculosis* and the development of new antibiotics is unlocking the secrets of its survival in the host cells. On the other hand, after development of the immune response, *M. tuberculosis* can be contained and/or eliminated in its human host. An understanding of how the immune system kills this pathogen is a key to development of more effective vaccines.

The first line of host defense against an infectious agent is its uptake and subsequent destruction by “professional phagocytes”. Cell biologists have compared the fate of *M. tuberculosis* with that of inert particles or non-pathogenic bacteria, after phagocytosis by macrophages (Fig. 1). When the latter are phagocytosed by a macrophage, they are usually found in a vacuole, the phagosome, which traffics along the endosomal-lysosomal pathway. Non-pathogenic bacteria are destroyed in the phagolysosome, by a combination of the low pH that activates the proteolytic enzymes, which enter these vacuoles, the respiratory burst resulting in the formation of

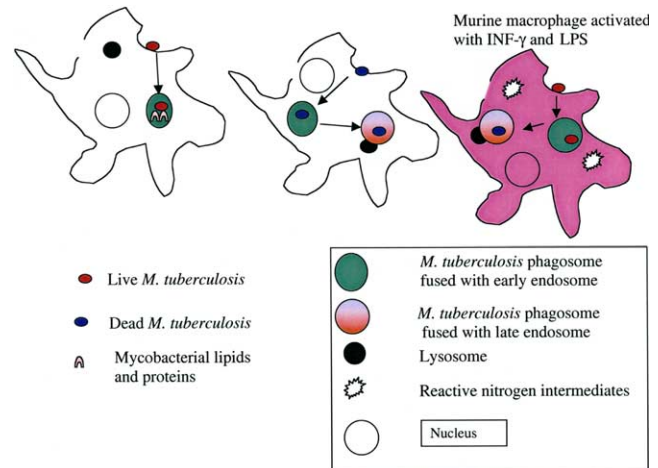


Fig. 1. Live *M. tuberculosis* resides in a specialized phagosome. Live *M. tuberculosis* enters the macrophage, and the phagosome fuses with early endosomes. Mycobacterial lipids and proteins are released into this phagosome and may be responsible for the inhibition of further trafficking. In contrast, vacuoles containing dead *M. tuberculosis* traffic to become late endosomes and fuse with lysosomes. If live bacteria enter an activated murine macrophage, which can kill *M. tuberculosis*, dead bacteria are found in late endosomal compartments that fuse with lysosomes.

superoxides, and, in murine macrophages, the synthesis of reactive nitrogen intermediates. The earliest observations that vacuoles containing live, but not dead, *Mycobacteria* fail to fuse with lysosomes were made in 1969 [1]. Studies describing the characteristics of the specialized vacuole in which live *M. tuberculosis* resides have revealed several interesting features. The mycobacterial phagosome fails to acidify below pH 6.3–6.5, presumably because of the lack of

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incorporation of a proton-pumping ATPase complex in the vacuolar membrane [2]. This low pH is necessary for the activation of hydrolases, present in the early endosomes, which are normally involved in the destruction of phagocytosed non-pathogenic bacteria. The mycobacterial phagosome also distinguishes itself by the retention of various cellular proteins, which are usually cleared from the phagosome as it matures. Some members of the Rab family of small GTPases, which confer fusion competence, are retained in the mycobacterial phagosome (Rab5), whereas others (Rab7) are excluded [3]. Rab7 has been implicated in interactions between phagosomes and late endosomal compartments (reviewed in Ref. [4]). It has been reported that live but not dead *M. bovis* BCG, which infects mouse macrophages, is held in a specialized compartment, that retains coronin 1 (also named TACO), a protein normally released prior to the maturation of the phagosome to lysosomes [5]. However, different results were described by Schuller et al. [6], who found that coronin was associated with the mycobacterial phagosome during uptake, but subsequently released. Another cellular protein, Gal-3, also accumulates in the mycobacterial phagosome [7]. Gal-3, a carbohydrate binding protein, can bind to mycobacterial phosphatidylinositol mannosides, which are among the various mycobacterial glycolipids released into the macrophage during infection [8]. However, the association of Gal-3 with the mycobacterial phagosome is not required for bacterial survival in the host macrophages, since *M. bovis* BCG grows normally in bone-marrow-derived macrophages from Gal-3-deficient mice [7]. A clearer picture of the significance of this result will be provided from the studies of *M. tuberculosis* growth in the Gal-3-deficient mouse.

The inhibition of *M. tuberculosis* phagosomal maturation was first proposed as a requirement for the survival of the pathogen in macrophages by D'Arcy Hart's group [9]. Subsequently, these researchers showed that when *M. tuberculosis* was pretreated with specific rabbit anti-mycobacterial antibody, prior to an infection of murine peritoneal macrophages, the phagosomes containing bacteria fused with lysosomes. However, contrary to their original hypothesis, this did not affect bacterial survival [10]. Although the original hypothesis is still favored by many cell biologists and repeated in the literature, there is no unequivocal evidence supporting it. In a recent study, *M. tuberculosis*, opsonized with specific anti-lipoarabinomannan (LAM) antibody, was used to infect the macrophages derived from peripheral blood monocytes from healthy humans [11]. Mycobacteria opsonized by the specific antibodies, but not by non-specific antibodies, induced elevated cytosolic Ca^{+2} levels in the macrophages, and this correlated with an increased maturation of the mycobacterial phagosome and a poor survival of the bacteria [11]. It should be noted, however, that the observed increases in the viable count of the bacteria, which were opsonized with non-specific antibodies in this study, did not correlate well with the known growth rate of *M. tuberculosis* in macrophages. Stimulation of murine macrophages with interferon γ

(IFN- γ) and lipopolysaccharide (LPS), a treatment that results in the killing of mycobacteria in these cells, also results in the acidification and maturation of the mycobacterial phagosome [12,13]. However, it is still unclear whether the observed trafficking of the mycobacterial phagosome, occurring after the activation of macrophages with LPS and IFN- γ , is the cause or effect of the killing of *M. tuberculosis* in the macrophage. Therefore, the hypothesis that *M. tuberculosis* manages to survive and grow in macrophages, because of its ability to halt phagosome-lysosomal fusion, remains attractive but still unproven.

Like all organisms, *M. tuberculosis* is expected to alter its program of gene expression when its environment changes, and its survival in macrophages should depend on such changes in gene expression. The mycobacterial phagosome is a dynamic structure [14]. Lipid-containing constituents of the mycobacterial cell wall, including LAM and phosphatidylinositol mannoside, are actively trafficked out of the phagosome and are released through exocytosis [15]. In response to the *M. tuberculosis* infection, the macrophage alters its program of gene expression [16,17], and a complicated process of cross-talk exists between the host and its pathogen. It is well known that activated macrophages can produce an oxidative burst, although this does not seem to affect *M. tuberculosis* viability in murine macrophages [18]. In this system, the activation of macrophages also induces the synthesis of inducible nitric oxide synthase, resulting in the production of nitric oxide and other reactive nitrogen intermediates, which are responsible for the killing of intracellular *M. tuberculosis* [18]. Since these conditions are potentially harmful to the infecting mycobacteria, they should induce an appropriate protective response. Therefore, the changing environment within the macrophage should elicit new programs of gene expression in the pathogen, and studies of these changes should provide an insight into how mycobacteria cope with the signals received from the host as well as what nutrients are available during infection. Since studies with other pathogens have shown that genes, which are upregulated during infection are often essential for pathogenicity [19,20], we can reasonably expect to learn equivalent lessons from such studies in *M. tuberculosis*. For this reason, many researchers studying the pathogenicity of *M. tuberculosis* have focused on studies of alterations in its gene expression during growth in macrophages compared with growth in broth. Many studies are based on the behavior of *M. tuberculosis* during infection of cell lines such as THP-1, which can be differentiated by phorbol esters into a macrophage-like state. Although these cells are certainly not the same as primary host cells, and are not activated, in many ways they behave like primary cells during infection by *M. tuberculosis*. They are induced to undergo apoptosis in a tumor necrosis factor-dependent manner during infection by mycobacteria, like human alveolar macrophages [21]. THP-1 cells bind mycobacteria in a similar way to human monocyte-derived macrophages, and the bacteria grow to the same extent [22]. *M. tuberculosis* does not traffic to late endosomes either in

primary macrophages or in THP-1 cells. Most of the studies with THP-1 cells do not compare the response of the pathogen to activated versus unactivated macrophages. Studies on transcriptional profiling of *M. tuberculosis* during infection of activated and unactivated macrophages are underway in several laboratories and this, of course, is the technology necessary for a global approach to gene expression.

Our discussion on the current state of knowledge in this field will be organized according to the types of genes of *M. tuberculosis* whose expression is upregulated in macrophages. The purpose of this review is not to list all the genes, which have been reported to be induced in these conditions, but rather to select those genes which can be placed in an intellectual context. Of course this will result in an incomplete picture, but we hope that the snapshot will be useful in conceptualizing how this important pathogen responds to its intracellular environment.

2. Genes controlling cell wall and surface-expressed proteins

Alterations of the bacterial cell surface during infection may be crucial for communication with the host cell and/or for survival of the bacteria. A good example is the change in the structure of LPS in *Salmonella typhimurium* during intracellular growth. This change is due to the upregulation by PhoP of genes controlling lipid A biosynthesis [23]. Lipid A is the major signaling component of LPS, which stimulates cytokine release in the host. Another example of alterations in the cell wall are the changes in the peptidoglycan structure of *S. typhimurium* growing within cultured cells [24]. It is logical to think that LPSs or other components of the cell surface may play a similar role in *M. tuberculosis* and, in fact, LAM has been implicated in such signaling [25]. In this connection, it is interesting to note that the transcripts of *fbpB*, a coding for mycolyl transferase (antigen 85B), an enzyme that transfers mycolates to trehalose [26] and to arabinogalactan [27], are increased after an infection of human monocytes. This was shown by SCOTS (selective capture of transcribed sequences) [28] and RT-PCR [29], and the protein levels of antigen 85B were also found to be higher after growth in the THP-1 human macrophage cell line [30]. Although Mariani et al. [31] obtained contradictory results measuring RNA transcripts by RT-PCR, their in vitro bacterial cultures were grown as a pellicle and harvested after 60 d of growth. Comparisons of RNA levels, using cultures grown under such conditions, with RNA levels from exponentially growing bacteria in macrophages may not be meaningful, since such an old culture is probably in a stationary phase. Although *fbpB* is not essential for the intracellular growth of *M. tuberculosis* [32], it is possible that it is required for full virulence in the animal model, and in fact, *fbpA*, a coding for another mycolyl transferase with similar activity, is required for growth in macrophage cell lines [32]. Incubation of *M. tuberculosis* with antisense RNAs directed against all the genes coding for mycolyl transferases caused a

significant loss in viability, thus, indicating this activity is essential and would be an excellent target for new antibiotics [33]. At the same time, mycolyl transferase B seems to be a protective antigen, since a strain of *M. bovis* BCG expressing this protein was found to be more effective as a vaccine against *M. tuberculosis* in guinea pigs than BCG itself [34]. One of the products of mycolyl transferase activity, trehalose 6,6'-dimycolate (TDM), may also be involved in the host response to infection by *M. tuberculosis* and is essential for virulence [35]. Recently, it was shown that delipidation of mycobacteria, causing the removal of TDM from the cell surface, results in a drastically altered pattern of cytokine expression and poor bacterial survival in murine bone marrow macrophages [36]. It should be noted, however, that the survival data consisted of a single point.

The gene coding for OmpATb, a porin of *M. tuberculosis*, was reported to be required for virulence in the mouse as well as for the growth in macrophages, although no complementation data are provided, such a conclusion is premature [37]. The gene was induced during the growth in macrophages as well as by low pH [37]. The authors speculate that this attribute may be related to the ability of *M. tuberculosis* to survive low pH in the mammalian host [37]. It is also possible that OmpATb is required for increasing permeability to small molecules and therefore for uptake of nutrients during growth in the host.

A promoter trap system (Fig. 2) was designed for *M. tuberculosis* [38], based on promoter-driven overexpression of *InhA*, the major target for isoniazid [39,40]. Selection of promoters differentially expressed in cultures growing in macrophages as compared to broth was based on the identification of clones, which were resistant to isoniazid during growth in human THP-1 macrophages but remained sensitive to isoniazid during growth on plates. Several *M. tuberculosis* genes were identified by this selection system; one group was predicted to code for proteins located in or required for the structure of the cell surface [38]. These are: Rv0102, Rv1171, Rv2120c, annotated as coding for integral membrane proteins; Rv2171, coding for a lipoprotein with a membrane lipid attachment site; Rv3717, coding for *N*-acetyl-muramoyl-L-alanine amidase; and Rv3237c, coding for a potassium channel protein.

3. PPE/PE families

Among the most interesting gene families found in the mycobacteria are the PE and PPE families. Eight percent of the *M. tuberculosis* genome is devoted to these genes, encoding acidic glycine-rich proteins [41], some of which have shown to be surface exposed [42,43]. Although none of the PE-PGRS (a sub-family of the PE group) proteins has N-terminal signal peptides, several end up in the cell envelope [42,43]. The field is rife with speculation on the functions of these proteins, including their functions to provide antigenic variation and interfere with immune responses [41], in addition to performing a purely structural role [42].

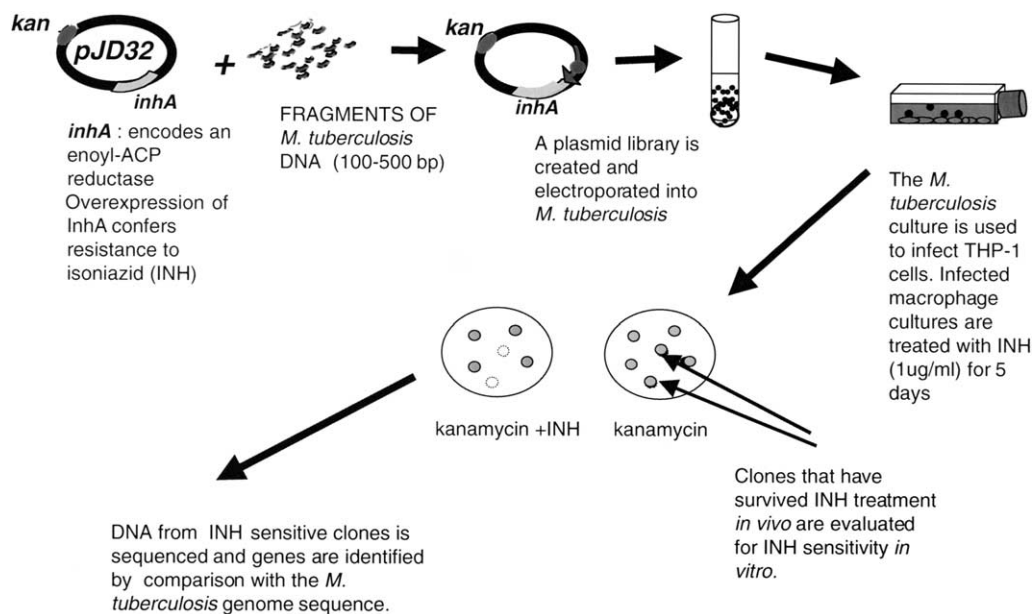


Fig. 2. Promoter trap for *M. tuberculosis*. A promoter trap plasmid was constructed by incorporating a cassette coding for kanamycin resistance (Kan) and a copy of *inhA* with no promoter. Random fragments of *M. tuberculosis* DNA were cloned upstream of *inhA*, and the resulting plasmid library was electroporated into *M. tuberculosis*. This library was used to infect THP-1 cells, and the infected cells were treated with isoniazid (INH) to select clones carrying promoters driving *inhA* expression. Clones surviving the treatment were isolated on plates without isoniazid and then screened for sensitivity to INH. INH-sensitive clones are presumed to carry promoters driving *inhA*, specifically when the bacteria are growing in mammalian cells but not during growth in culture medium.

A GFP promoter trap system identified a member of the PE-PGRS subfamily of *M. tuberculosis* as upregulated in macrophages [44]. Using a similar technology, two *M. marinum* orthologs of *M. tuberculosis* PE-PGRS genes were identified [45]. These genes were specifically expressed in the J774 murine macrophage cell line and in granulomas from the livers and spleens of infected frogs. A mutant strain containing an inactivated gene coding for one of these proteins was attenuated in frogs and could not grow in murine macrophages. The latter observation suggests that antigenic variation, which would only be manifested in the whole animal, is not the main function for this gene in *M. marinum* [45]. The promoter trap system discussed above identified several genes, i.e. Rv0977 (PE-PGRS16), Rv1361c (PPE19) and Rv1840c (PE-PGRS34), of these extensive *M. tuberculosis* gene families as upregulated in THP-1 macrophages [38].

4. Genes coding for secreted proteins

Some of the secreted proteins of *M. tuberculosis* are very antigenic, presumably because they can be presented to T cells after secretion into the milieu of the phagosome. A family of immunodominant small proteins with about 22 members, the “ESAT-6” family, is recognized by the sera of a high proportion of tuberculosis patients [46]. A subunit vaccine made with a fusion protein of the ESAT-6 antigen with antigen 85B, is as protective as BCG against challenge with *M. tuberculosis* in mice [47]. Increased expression in macrophages of *fbpB*, coding for antigen 85B was mentioned above, but promoters of several genes encoding other anti-

gens have also been reported to be induced during growth in macrophages. The *M. leprae* promoter for the gene encoding the 18-kDa antigen, a homolog of the 16-kDa antigen of *M. tuberculosis*, was shown to be upregulated in a recombinant strain of BCG, during infection of the J774 macrophage cell line [48]. These workers used *lacZ* as a reporter gene, but expressed in Miller units for the broth cultures and in arbitrary luminescent units for the macrophage-grown bacteria [48], making it difficult to assess the data. In addition, there was no normalization for the number of bacteria, so the quantification was questionable. Several other workers have shown that *hspX*, encoding the 16-kDa antigen, is upregulated during the growth of *M. tuberculosis* in THP-1 macrophages, by quantifying the proteins with two-dimensional electrophoresis [49], by GFP-reporter fusions [50], or RT-PCR [38]. There is a conflicting report that the *hspX* RNA levels decrease during growth in human monocytes [29]. There is no apparent explanation for the discrepancy between these published results other than that the conditions in human monocytes may be very different from those in other macrophages and therefore result in lower expression of *hspX*. A strain with mutated *hspX* does not grow in primary mouse bone-marrow-derived macrophages or in THP-1 cells, supporting the view that this upregulated gene is also required for virulence [50]. Unfortunately, Yuan et al. do not present data showing that complementation of the mutant strain restores the ability to grow in macrophages. The *mig* gene of *M. avium*, postulated to code for a secreted protein [51], is only transcribed when the bacilli are growing in macrophages or under acidic conditions [52].

5. Genes controlling iron acquisition

Since the levels of iron available to bacterial pathogens are low in the mammalian host [53], it is not surprising that *M. tuberculosis* also has a system for iron acquisition, which could be upregulated during infection. *Yersinia*, *Salmonella*, and *Legionella* cannot survive in the mammalian host without functional iron acquisition systems [54–56]. Indeed, the RNA levels of *mbtB* and *mbtI*, measured by quantitative RT-PCR, are higher in cultures of *M. tuberculosis* during growth in macrophages compared with broth cultures [57]. *mbtB* and *mbtI* are part of the operon controlling the biosynthesis of the siderophore mycobactin [58] and are regulated by IdeR, the main repressor of iron-regulated genes in *M. tuberculosis* [59]. *mbtB* seems to be required for growth in THP-1 macrophages, although no complementation data are presented, the conclusions reported must be regarded as preliminary [60]. The fact that the expression of *mbtB* and *mbtI* is also induced by low iron levels during growth in culture medium [57] strongly suggests that iron levels available to *M. tuberculosis* are low in the macrophage. Supporting this idea is the observation that an *M. marinum* gene with similarity to the iron-regulated *Pseudomonas* *pvdD* was upregulated during the growth in macrophages [61].

6. Genes encoding regulators

Regulators of gene expression include sigma factors and DNA binding proteins, which repress or activate transcription of their target genes. Transcription of genes coding for these regulators may also be regulated. *furB*, a putative divalent metal uptake regulator, was upregulated in *M. bovis* BCG when growing in the human monocytic cell line, THP-1 [62]. Related proteins of the Fur family in other bacterial systems are global regulators for various virulence factors as well as iron acquisition and oxidative- and acid-stress responses [63]. In *M. tuberculosis*, *furB* transcription may respond to various signals originating in the macrophage environment, including low metal levels. In this connection, it is interesting that IdeR, a repressor of iron-regulated genes [59], was also reported to be upregulated in *M. bovis* BCG growing in the same macrophage cell line, in experiments using *lacZ* reporter fusions to *M. tuberculosis* promoters [64]. This is unexpected because, as mentioned above, several iron-regulated genes are themselves upregulated during the growth of *M. tuberculosis* in macrophages [57]. This apparent contradiction could be explained either by differences between transcriptional regulation during intracellular growth of *M. bovis* BCG and *M. tuberculosis* or by the hypothesis that the function of IdeR is more complex than a simple repression of iron-regulated genes. In fact, IdeR is an essential protein in *M. tuberculosis* [59] and is a positive regulator of several genes [57,59].

The transcriptional regulator (TetR/AcrR family) Rv0302 as well as the stress-response protein encoded by Rv0440

(GroEL2) was also identified by the *lacZ* fusion assays as upregulated during intracellular growth [64].

prpA, encoding a putative bacterial two-component transcriptional activator, and *prpB*, coding for the cognate sensor kinase, as well as *sigE* and *sigH*, coding for alternative sigma factors, which are required for virulence [65,66], were shown by SCOTS to be upregulated in macrophages [28]. A GFP-fusion promoter trap system for the selection of genes induced in macrophages identified an *M. marinum* gene, *map25*, with 96% homology to Rv3416 of *M. tuberculosis* [45]. This gene codes for a protein that is very similar to WhiB3, a transcriptional activator of early sporulation in *Streptomyces coelicolor* [67]. *whiB3* was knocked out in *M. tuberculosis*, and the mutant strain, although it grows normally in mice, does not cause the same pathology as the wild-type strain and manifests an attenuated phenotype; mice or guinea pigs infected with this mutant strain show a prolonged survival compared with animals infected with the wild-type strain [68]. WhiB3 interacts with the major sigma factor of *M. tuberculosis*, RpoV (SigA), presumably activating the transcription of specific genes required for pathogenesis [68]. It is interesting that a strain of *M. bovis* with an identical mutation in *whiB3* was unable to grow in guinea pigs [68]. The promoter trap system using *inhA*, described above, identified Rv0549c, Rv2009 and Rv3321c, putative transcriptional regulators by virtue of their helix-turn-helix DNA binding motifs, as upregulated in THP-1 macrophages, and quantitative RT-PCR verified that the level of Rv3321c RNA was higher in macrophage-grown cultures of *M. tuberculosis* than in broth-grown cultures [38].

7. Genes encoding enzymes involved in lipid metabolism

Early physiological studies, based on the measurements of respiratory response in the Warburg apparatus, suggested that *M. tuberculosis* isolated from infected mouse lungs was metabolically distinct from *M. tuberculosis* grown in broth culture [69]. Bacteria from the mouse lungs respired in response to fatty acids but not to carbohydrates or other carbon sources, whereas bacteria grown in broth culture respired in response to all carbon sources. These early experiments were subject to error because the prolonged techniques used for the separation of the bacteria from the lung tissue could themselves cause changes in bacterial metabolism. However, recent studies have demonstrated that lipid metabolism is essential for the virulence of *M. tuberculosis*, confirming the earlier observations. This microorganism is unique in that its genome encodes more than 250 distinct proteins annotated to be involved in fatty acid metabolism [41]. Unlike other bacteria, *M. tuberculosis* has at least two fatty acid biosynthetic systems, FASI and FASII that are responsible, respectively, for the biosyntheses of shorter chain and for long chain fatty acids, which are precursors for mycolic acids typical of mycobacteria. These specialized fatty acids can be esterified to arabinogalactan and make up a major portion of the cell

wall. Other cell wall lipids of significance include phthiocerol dimycocerosate, which is essential for virulence [70,71], mycolates esterified to trehalose (TDM), mentioned above, also essential for virulence [35], and methyl-branched fatty acids (reviewed in Ref. [72]). cDNA-RNA subtractive hybridization using RNA from *M. bovis* BCG growing in the human macrophage cell line THP-1 or in broth culture, identified *mas* and *fadD28* as upregulated during intracellular growth [62]. These are linked genes required for biosynthesis of mycocerosic acid (*mas*), a methyl-branched long chain fatty acid, and acyl coenzyme A synthase (*fadD28*), involved in esterification of this acid to phthiocerol to generate phthiocerol dimycocerosate. Recent work on the regulation of these two important genes [73] shows the presence of an enhancer-like element about 580 bp upstream of the *mas* promoter, and perhaps this element is involved in the regulation during growth in macrophages.

Isocitrate lyase, part of the anapleurotic pathway for the replenishment of tricarboxylic acid cycle intermediates, is an enzyme required for growth on fatty acids. This protein was shown by 2D electrophoresis to be upregulated during growth in macrophages [74] and, consistent with these results, the SCOTS technique showed that *aceA*, coding for this enzyme, was expressed at higher levels in macrophages than during growth in broth culture [28]. Isocitrate lyase is required for the utilization of acetate, a product of fatty acid degradation in *M. smegmatis* [75], and presumably this is also true for *M. tuberculosis*. Further work using GFP-fusions confirmed the *aceA* upregulation in macrophages and also demonstrated that the gene was essential for the persistence of *M. tuberculosis* in mice [75]. It has long been known that bacteria from stationary or microaerophilic cultures produce higher levels of isocitrate lyase than bacteria in log-phase cultures [76,77]. However, the new information about the activity of the gene in macrophages and its role in virulence highlights the significance of fatty acid degradation in the ability of *M. tuberculosis* to cause disease. The fact that the mutant strain was only attenuated in activated bone marrow macrophages and the attenuation in mice was only manifested after a two-week infection indicates that isocitrate lyase is only required after the induction of cellular immunity. This implies a major overhaul of the bacterium's metabolism in favor of fatty acid degradation in response to the induction of cellular immunity. The promoter trap system, discussed above [38], identified several genes involved in fatty acid metabolism, namely *aceA*, *fadA4*, *echA19*, and *fadA5*. In addition to these, other genes which could be involved indirectly in fatty acid metabolism were *ephF*, Rv0610c, Rv1144, and Rv1774, encoding, respectively, epoxide hydrolase, monooxygenase, alcohol dehydrogenase, and oxidoreductase. In addition, the promoter trap system identified *pckA*, encoding phosphoenol carboxykinase and *eno*, coding for enolase. Quantitative RT-PCRs demonstrated that *aceA*, *echA19*, *fadA4* and *pckA* were indeed upregulated during the growth in THP-1 macrophages [38]. *fadB4* was also identified as upregulated [44].

8. Conclusions

We can draw several conclusions from our survey of the literature on *M. tuberculosis* gene expression in macrophages. First, in agreement with earlier studies on *M. tuberculosis* isolated from mouse lungs [69], many genes involved in fatty acid metabolism are upregulated during growth in macrophages. This highlights the significance of these pathways for *M. tuberculosis* during infection. In addition, genes required for the modeling of the cell surface, including components of the cell wall, are upregulated. The cell surface is at the interface with the environment and therefore may have to be modified after infection. Several regulators, of unknown function, are also transcribed at higher levels during infection and this may reflect the need for an expression of new regulons in response to the host environment.

Many genes have been reported as upregulated in macrophages, including some of totally unknown function, but we have focused on those which fall into identifiable groups, the analysis of which can shed some light on the pathogenic process. None of the methods used so far, such as SCOTS, 2D protein electrophoresis, various promoter traps or RT-PCR, have provided a complete picture, and this is also true for work done with other pathogens. Recent work on another intracellular pathogen, *Brucella suis*, has shown that most genes required for survival in THP-1 cells are involved in what are generally considered housekeeping functions, such as amino acid biosynthesis and metabolism of DNA/RNA, sugars, nitrogen and lipids [78]. The pattern of *M. tuberculosis* gene expression in macrophages does not lead to similar conclusions on the genetic requirements for survival of this pathogen. Instead, genes coding for various regulators, iron acquisition, cell surface components and fatty acid metabolism are highlighted as important.

Comparative transcriptional profiling of *M. tuberculosis* growing in macrophages and in broth should provide a more complete understanding of the physiology of this pathogen during its intracellular life cycle. A description of the regulons controlling growth during infection is key to uncovering essential pathways for the survival of *M. tuberculosis* growing in its mammalian host and to design new strategies for diagnosing and curing people infected with this ancient pathogen. Of course, the best cure is preventing disease, and this depends on improving the standard of living in the world.

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