PROSPECTS
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Ins and Outs of *Mycobacterium tuberculosis* PPE Family in Pathogenesis and Implications for Novel Measures Against Tuberculosis

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ABSTRACT

Mycobacterium tuberculosis is the most successful pathogen with multiple mechanisms to subvert host immune response, resulting in insidious disease. A unique *Mycobacterium* antigen family termed PPE (Pro-Pro-Glu) has long been widely speculated as ''molecular mantra'' to escape host immunity. Members of this family are characterized by a conserved N terminal and a variable C terminal. This family associated closely with ESAT-6(ESX) secretion system and largely located in cell wall or cell membrane. The expression of PPE protein is temporally regulated, and highly expressed during *M. tuberculosis* persistence. Importantly, the distribution of PPE family is so far limited to *Mycobacterium* genus, prevalent among pathogenic *Mycobacterium* species. It is tempting to explore this family due to its potential in the latency and reactivation of *M. tuberculosis*. The evolution, structure, and functions of most PPE proteins remain elusive. The understanding of these questions will deepen our appreciation of the pathogenesis of *M. tuberculosis* and accelerate novel anti-TB measures discovery. J. Cell. Biochem. 113: 1087–1095, 2012.

KEY WORDS: *M. tuberculosis*; PPE FAMILY; PATHOGENESIS; IMMUNE EVASION; VACCINE

Tuberculosis continues to be one of the most prevalent and deadly infectious diseases. About one third of the world population is latently infected with *M. tuberculosis*, and in 2008 an estimated 1.3 million people died of tuberculosis and an estimated 9.3 million people developed the active tuberculosis worldwide. The lengthy duration of treatment with a combination of three to four antibiotics, the poor patient compliance, shortage of novel drugs, emerging of multi-drug resistant (MDR), and extensive drug resistant (XDR) *M. tuberculosis* strains, increase incidence of HIV co-infection, and the unreliable drug supply synergistically exacerbated the scenario [Luthi and Diacon, 2011; Venkatesh et al., 2011]. Therefore, effective new measures are urgently needed to control TB. The biology of *M. tuberculosis* unique genes might offer unprecedented opportunity.

PE (Pro-Glu) and PPE (Pro-Pro-Glu) are two-gene families account for about 10% of the *M. tuberculosis* genome, with 99 and 69 members, respectively [Cole et al., 1998]. The name of PE and PPE is derived from N-terminal Pro(P)-Glu(E) and Pro(P)-Pro(P)-Glu(E) residues [Cole and Barrell, 1998]. They have a conserved N-terminal approximately 110 and 180 amino acid residues and a C-terminal varying significantly with sequences and sizes [Bottai and Brosch, 2009]. PE is characterized by multiple copies of PGRS (polymorphic GC-rich repetitive sequences) and PPE bears a MPTR (major polymorphic tandem repeats) in their C terminal [Hermans et al., 1992; Poulet and Cole, 1995]. Some previous excellent reviews have covered the origin, physiological role, and spatiotemporal regulation of some well-characterized PE family proteins [Brennan and Delogu, 2002; Tian and Jian-Ping, 2010]. In this review, we aim to sum up the evolution, structure, and function of another important antigen family (PPE), as well as their implications in novel vaccines (Table I) and diagnostics against tuberculosis.

Additional Supporting Information may be found in the online version of this article.

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TABLE I. The Features of Some Well-Characterized PPE Family Members

PPE	Gene	Vaccine and/or biomarker	Immunoreactivity	Reference
PPE17	Rv1168c	Biomarker in clinically active TB	Stronger immunoreactivity than PPD, Hsp60, or ESAT-6	Khan et al., (2008)
PPE18	Rv1196	A potential T cell antigen	Elicite strongly the proliferation of T-cell and IFN-γ responses than PPD	Dillon et al. (1999), Nair et al. (2009, 2011)
PPE34	Rv1917c	A potential T cell antigen	Mediates the secretion of IL-4, IL-5, and IL-10	Bansal et al. (2010)
PPE41	Rv2430c	A potential B cell antigen	Strong B-cell response compared to Hsp10 or PPD	Choudhary et al. (2003, 2004)
PPE42	Rv2608	A potential B cell antigen	Predominantly humoral response	Chakhaiyar et al. (2004)
PPE44	Rv2770c	Subunit anti-TB vaccine and biomarkers of acutely, chronically and latently infectious stages	Strong cellular and humoral immune responses than BCG	Cuccu et al. (2011), Romano et al. (2008)
PPE55	Rv3347c	Biomarker in all infection stages	Anti-PPE55 antibodies response	Singh et al. (2005)
PPE57	Rv3425	rBCG::Ag85B-Rv3425 subunit vaccine and biomarker	Produce higher IgG2a and IFN-γ than rBCG::Ag85B	Wang et al. (2009)
PPE68	Rv3873	T-cell antigen	Trigger higher IFN-γ than PPD	Okkels et al. (2003)

THE DISTRIBUTION AND EVOLUTION OF PPE FAMILY

There are 69 PPE members in *M. tuberculosis*, up to 6% of the coding capacity of the whole-genome [Cole et al., 1998; Brennan and Delogu, 2002]. Previous reports about some repetitive proteins homologous to PPE in *Corynebacterium* and *Nocardia farcinica* genomes might result from unspecific alignment of repetitive regions, due to absence of typical PPE motif in their N terminal [Cerdeno-Tarraga et al., 2003; Ishikawa et al., 2004]. Comparative genomic analysis suggests that PPE proteins were largely limited to *Mycobacterium* genus [Gey van Pittius et al., 2006], especially enriched in pathogenic *Mycobacterium* species (Fig. 1), such as *M. tuberculosis*, *M. bovis*, *M. ulcerans*, *M. marinum*, and *M. kansasii*, suggestive of unique roles in the virulence, pathogenesis, and persistence of *Mycobacterium*.

The evolution of PPE family was presumably associated with ESAT-6 (esx) gene cluster [Gey van Pittius et al., 2006], encoding the ESAT-6 family T-cell antigen secretion system [Guinn et al., 2004]. PE and PPE genes were initially inserted into the ESAT-6 (esx) gene cluster region 1, subsequently duplicated along with the ESAT-6 regions and expanded. Every major duplication event was followed by numerous minor subduplications. The evolution of PE_PGRS (PE) and PPE_MPTR (PPE) subfamily is relatively a recent event, which occurred at the defined evolutional branching points of *Mycobacterium*. PE_PGRS emerges after the divergence of the *M. avium* complex and *M. leprae*. PPE_MPTR evolves before the divergence of *M. marinum*/*M. ulcerans* and the *M. tuberculosis* complex [Gey van Pittius et al., 2006]. The C-terminal PGRS repeat elements (TTGCCGCCGTTGCCGCCG) of some PE genes resembles the C-terminal MPTR repeat sequence (GCCGGTGTTG) of the PPE genes [Hermans et al., 1992; Poulet and Cole, 1995]. In addition,

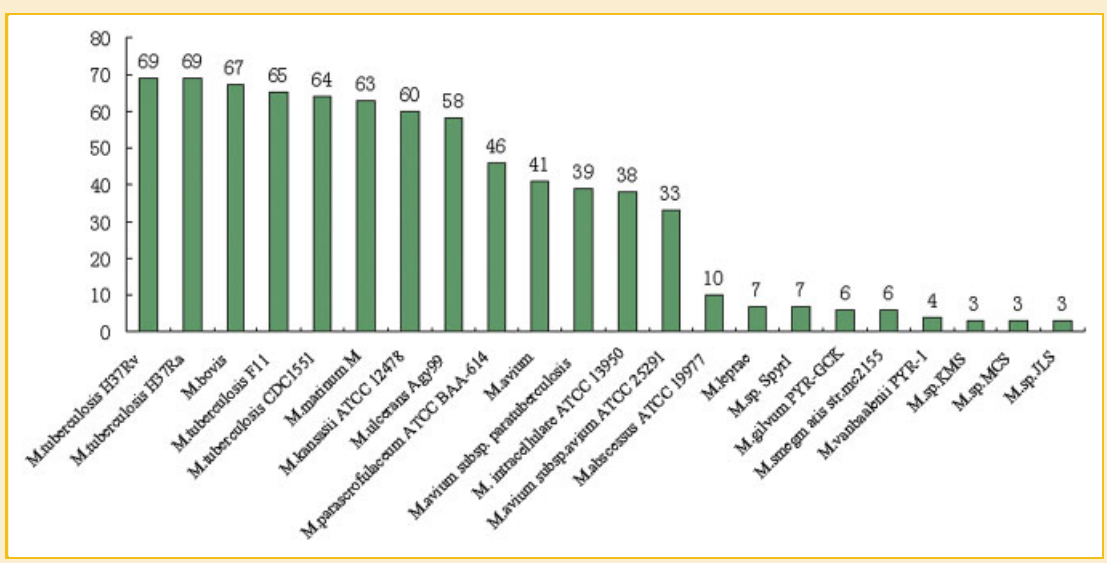

Fig. 1. The distribution of PPE proteins among *Mycobacterium*. The X- and Y-axis represent different *Mycobacterium* strains and corresponding numbers of PPE proteins, respectively. Complete sequence alignment was performed with the amino acids of PPE orthologs from all GenBank database available *Mycobacterium* genomes (http://www.ncbi.nlm.nih.gov/). The results were generated by BLAST (http://blast.ncbi.nlm.nih.gov/). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

11 of the predicted 69 PPE proteins lack the characteristic N-terminal PPE motif. Six of these PPE6/Rv0305c, PPE57/Rv3425, PPE58/Rv3426, PPE59/Rv3429, PPE63/Rv3539, and PPE69/Rv3892c result from a substitution in one of the two-proline residues in the conserved motif. The upstream regions of another five proteins (PPE5/Rv0304c, PPE7 /Rv0354c, PPE39/Rv2353c, PPE47/Rv3021c, and PPE66/Rv3738c) were disrupted by either IS6110 insertion or apparent frameshift mutations [Gey van Pittius et al., 2006]. Together, these data imply that the evolution of the two gene families (PE and PPE) may through insertion/deletion, which serve as a major source of antigenic variation [Talarico et al., 2008].

THE PRIMARY STRUCTURE OF PPE PROTEINS

Most members of PPE family have a conserved N-terminal domain followed by a C-terminal domain. Based on the motifs and repeat copy numbers of C terminal sequence, this family can be divided into four subfamilies [Adindla and Guruprasad, 2003]. The first is the 24 members PPE-SVP subfamily, which is the largest one bearing a typical motif Gly-X-X-Ser-Val-Pro-X-X-Trp between position 300 and 350 [Adindla and Guruprasad, 2003; Gey van Pittius et al., 2006]. The second subfamily is the PPE_MPTR (Major Polymorphic Tandem Repeat) subfamily characterized by multiple repeats (Asn-X-Gly-X-Gly-Asn-X-Gly) in its C-terminal, encoded by a consensus repeat sequence GCCGGTGTTG that is spaced by 5 bp nucleotides [Hermans et al., 1992; Cole and Barrell, 1998]. The third subfamily is PPE-PPW which has a conserved 44 amino acid residue comprising of highly conserved Gly-Phe-X-Gly-Thr and Pro-X-X-Pro-X-X-Trp motifs at its C terminus [Adindla and Guruprasad, 2003; Gey van Pittius et al., 2006]. The last subfamily has 12 members with a low homology at the C-terminal sequence [Gey van Pittius et al., 2006].

The C-terminal domain (225 amino acids residues) of PPE proteins (PPE_MPTR) resembles PE proteins (PE_PGRS) termed "PE-PPE domain" [Adindla and Guruprasad, 2003], which contains a GxSxG/S motif at N-terminal and a serine α/β hydrolase fold (a central β-sheet flanked by α-helices on either side) with specific esterase, lipase, or cutinase activity [Sultana et al., 2011]. The structure comprises a lid insertion with a closed conformation and a solvent inaccessible active site. The oxyanion hole that stabilizes the negative charge on the tetrahedral intermediate has been identified [Sultana et al., 2011]. This has expanded the extents of serine hydrolases, which are necessary to maintain the impermeable cell wall and virulence of *Mycobacterium*. Circular dichroism (CD) spectrum and web-based program K2D demonstrated that recombinant Rv2430c protein contains 81% α helical and 19% random coil, consistent with the in silico predictions of a predominant α helical [Choudhary et al., 2004]. The relative molecular weight of recombinant Rv1168c protein was about 51.5 kDa. Secondary structure of Rv1168c has about 34.4% α helix, 33.7% β turn, and 31.9% random coil [Yu et al., 2010]. The heterologous expression of PPE is difficult, therefore, only few PPE protein crystal structures are available. A case in point is the PE/PPE complex solved by David Eisenberg et al. [Strong et al., 2006], which shows that the PE and PPE proteins align along an extended apolar interface to form a four-α-helical bundle, where two of four α-helices are formed by the PE protein and two by the PPE protein, respectively (Fig. 2). The structure and function of proteins are usually consistent. More structures of PPE family proteins will shed more lights on their function.

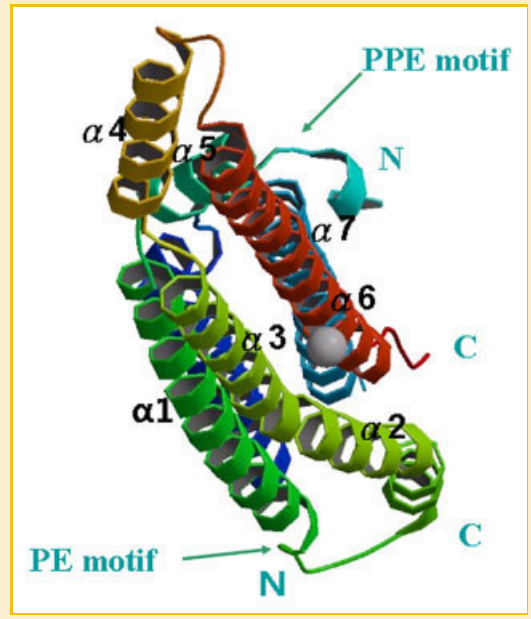

Fig. 2. The secondary structure of Rv2430c-Rv2431c complex (PDB 2G38) (Strong et al., 2006). The complex is composed of seven α-helices, Two α-helices of the PE protein interact with two helices of the PPE protein to form a four-helix bundle. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

THE EXPRESSION AND REGULATION OF PPE GENES

Some PPE family genes are involved in the *M. tuberculosis* persistence and highly expressed during multiple conditions, such as high-iron [Rodriguez et al., 2002], infected macrophage [Schnappinger et al., 2003], hypoxia [Park et al., 2003], nonreplicating persistence, and stationary phase [Voskuil et al., 2004b], SDS [Manganelli et al., 2001], diamide [Manganelli et al., 2002], and diethylenetriamine/nitric oxide adduct (DETA/NO or DNO) [Voskuil et al., 2003]. The PE/PPE might be the crucial for the antigen variation during host infection, since 128 of the 169 PE/PPE genes and approximately two-third of the PPE genes (Fig. 3) are differentially expressed under 15 conditions as previously described and others including hydrogen peroxide (H_2O_2), potassium cyanide (KCN), carbonyl-cyanide 3-chlorophenylhydrazone (CCCP), ethambutol, palmitic acid, starvation, and heat shock (45°C) have clearly manifested this diversity [Voskuil et al., 2004a]. No expression of PPE5/Rv0304c, PPE7/Rv0354c, PPE9/Rv0388c, PPE24/Rv1753c, PPE49/Rv3125c, PPE64/Rv3558, PPE65/Rv3621c, PPE66/PPE66, PPE69/Rv3892c can be detected under all tested circumstances. Importantly, seven PPE proteins (PPE17/Rv1168c, PPE18/Rv1196, PPE32/Rv1808, PPE33/Rv1808, PPE50/Rv3135, PPE51/Rv3136, PPE60 /Rv3478) as highlighted in orange are regulated under at

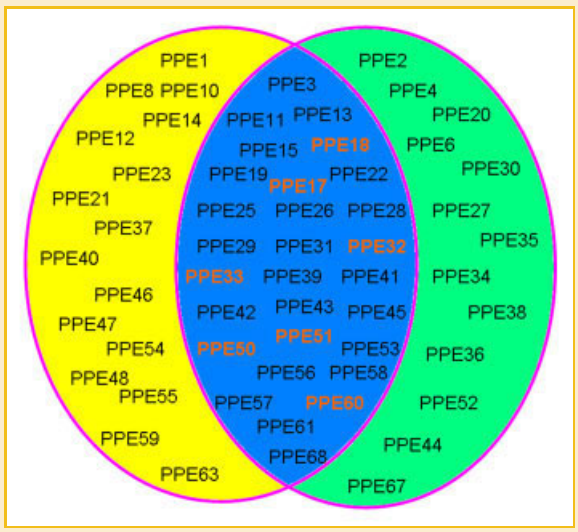

Fig. 3. The differential expression of 69 PPE proteins under 15 different conditions as described in the text. PPE proteins are induced only (yellow), induced in some condition but repressed in another conditions (blue region) and repressed only (green region), respectively. Among these, seven PPE proteins highlighted in orange are regulated by at least five treatments. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

least five conditions, suggesting important roles in the pathogenesis of *M. tubercurosis* and deserve further study. Recent study has shown that the expression patterns of PPE_MPTR subfamily genes vary with each member and differentially regulated during mice infection [Soldini et al., 2011]. Four of 10 PPE_MPTR genes are constitutively expressed and maintained at similar level, including PPE_MPTR10/Rv0442c, PPE_MPTR13/Rv0878c, PPE_MPTR40/Rv2356c, and PPE_MPTR62/Rv3533c. The expression of another four genes is inducible, including PPE_MPTR34/Rv1917c, PPE_MPTR35/Rv1918c, PPE_MPTR6/Rv0305c, and PPE_MPTR53/Rv3159c. Two PPE proteins (PPE_MPTR 16 and PPE_MPTR12/Rv0755c) cannot be detected during different infection stages. Most PPE genes give a poor expression or expressed in insoluble or unfolded forms [Strong et al., 2006]. Some unknown factors assisting the expression and correctly fold of PPE proteins might exist. PE/PPE genes are regulated pairwise, PE5/PPE4 (Rv0285/Rv0286), PE13/PPE18 (Rv1195/Rv1196), PE15/PPE20 (Rv1386/Rv1387), and PE25/PPE41 (Rv2431c/Rv2430c) are down-regulated while PE11/PPE17 (Rv1169c/Rv1168c) is up-regulated during nutrient starvation [Betts et al., 2002]. Interestingly, either PE25/Rv2431c or PPE41/Rv2430c alone can be only found *E. coli* inclusion bodies, when co-expressed, both proteins are soluble [Tundup et al., 2006]. This suggests that the interactions between PE and PPE proteins may mutually promote the correct fold or PE and PPE proteins may serve as reciprocal chaperones.

THE SUBCELLULAR LOCALIZATION OF PPE FAMILY PROTEINS

Most pathogen depends on specific secretion system such as Type VII or ESX to deliver their virulence factors into the host. *M. tuberculosis* genome has five ESX members, namely ESX-1 (Rv3866-Rv3883c), ESX-2 (Rv3884c-Rv3895c), ESX-3 (Rv0282-Rv0292), ESX-4 (Rv3444c-Rv3450c), and ESX-5 (Rv1782-Rv1798) [Gey Van Pittius et al., 2001; Brodin et al., 2004]. ESX-1 locates in RD1 region, which is responsible for the secretion of many substrates including important T cell antigens ESAT-6 and CFP-10. The latter two are associated with virulence and pathogenesis [Samten et al., 2009]. The virulence factors secreted by ESX-1 are recognized by multiple cytosolic AAA ATPases [DiGiuseppe Champion et al., 2009]. Esx-3 is a specialized secretion system essential for mycobactin-mediated iron acquisition [Siegrist et al., 2009]. PPE_MPTR subfamily might originate from PPE genes within the ESX-5 cluster, suggestive of the function link of ESX-5 with the recent expansion of PPE proteins [Gey van Pittius et al., 2006]. *M. marinum* ESX-5 mutant was defective in spreading to uninfected macrophages and the secretion of PPE41 (Rv2430c) [Abdallah et al., 2006], suggesting a pathogenic *Mycobacterium*-specific distribution of ESX-5 secretion apparatus.

The subcellular localization of PPEs might be of functional significance. For example, cell wall associated ones might be important in mediating the host–pathogen interaction. High-throughput proteomics approaches have demonstrated that cell wall/surface localization is a characteristic of several PE/PPE proteins [Sani et al., 2010]. PPE-MPTR subfamily may be cell wall associated and may play a role in the transmission of *Mycobacterium* through binding to host cell receptors [Doran et al., 1992]. PPE34/Rv1917 is hydrophobic and associates with cell wall, largely surface-exposed [Sampson et al., 2001]. The distribution of PPE36/Rv2108 is limited among *M. bovis* BCG and clinical isolates of *M. tuberculosis* and localized within cell membrane [Le Moigne et al., 2005]. PPE68/Rv3873 is also predominantly associated with the *Mycobacterium* cell wall [Okkels et al., 2003]. However, whether all members of PPE family are associated with cell well or secrete outwards remains to be determined. It was reported that PE and PPE domains of slow-growing *Mycobacterium* function as the signal essential for secretion of LipY (triacylglycerol lipase) via the ESX-5 system. These PE and PPE domains are removed upon translocation [Daleke et al., 2011].

THE FUNCTION OF PPE PROTEINS

PPE proteins are unique to *Mycobacterium* and some members of PPE family play important role in the pathogenesis of TB by regulating fatty acid metabolism and *Mycobacterium* virulence (Fig. 4), though little is known on their specific roles and functions.

PPE AFFECTS THE GROWTH OF *M. tuberculosis* DURING INFECTION

Transposon mutagenesis results have shown that PPE46/Rv3018c, PPE4/Rv0286, PPE12 /Rv0755c, PPE24/Rv1753c, PPE50/Rv3135, and PPE54/Rv3343c are essential for *M. tuberculosis* in vitro growth [Sassetti et al., 2003]. PPE31/Rv1807 and PPE68/Rv3873 are specifically required for *Mycobacterium* growth in vivo during infection of mice [Sassetti and Rubin, 2003]. In addition, PPE29/Rv1801 and PPE47/Rv3021c might be crucial for intracellular

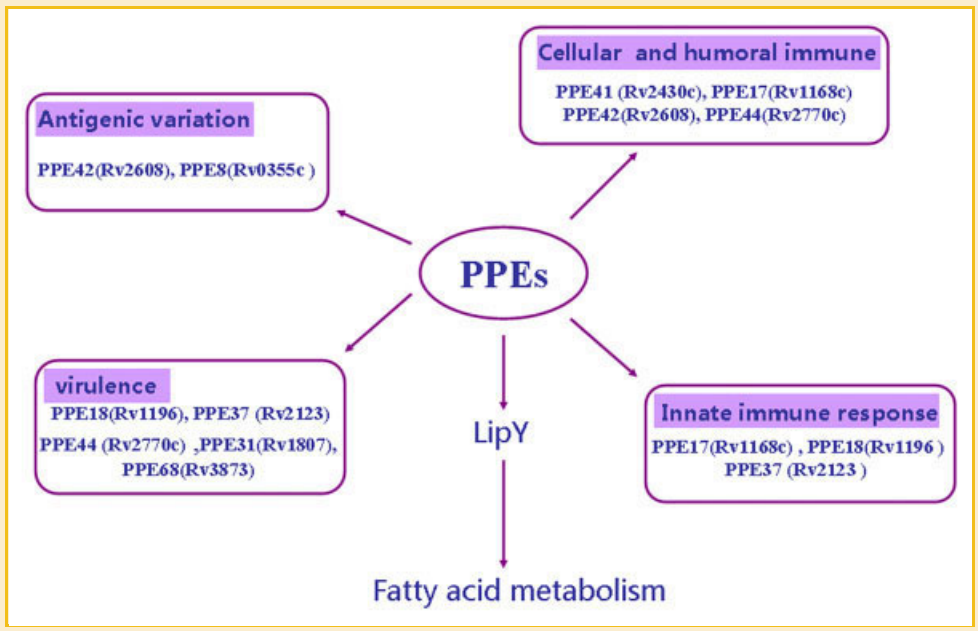

Fig. 4. The functions of some well-documented *M tuberculosis* PPEs. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

survival and endothelial-cell invasion, since they are up-regulated at least eightfold in human brain microvascular endothelial-cell-associated TB [Jain et al., 2006]. Some PPE proteins are up-regulated by multiple stresses, especially nutrition starvation, and nonreplicating conditions (Supplementary 1) which are closely relevant to the persistence of *M. tuberculosis*.

PPEs PLAY AN IMPORTANT ROLE IN *M. tuberculosis* VIRULENCE

Microarray and real time quantitative PCR analysis showed that the deletion of Rv0485 can decrease the expression of gene pair PE13/Rv1195 and PPE18/Rv1196, resulting in the attenuation of *M. tuberculosis* virulence and reduced secretion of pro-inflammatory cytokines by infected murine macrophages [Goldstone et al., 2009]. PPE41/Rv2430c induced in *M. tuberculosis* IdeR mutant strain may have an important role in the pathogenesis of *M. tuberculosis* [Rodriguez et al., 2002]. Ms_PPE37/Rv2123 can significantly lower the production of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) in the infected macrophages, also the transcription of nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK), and MAPK/p38 [Daim et al., 2011], suggesting a role of PPE37/Rv2123 in host immune evasion through interfering with the pro-inflammatory cytokines. Whether these PPEs function alone or synergistically with other factors remain to be determined. This is a very interesting and fruitful field to discover the intricate interaction between host and pathogen.

PPEs SERVE AS LIPASE INVOLVED IN FATTY ACID METABOLISM

The N-terminal of PE_PGRS63 ($LipY_{tub}$, encoded by Rv3097) is homologous with the proline glutamic acid polymorphic GC-rich repetitive sequences protein family of *M. tuberculosis*. The C-terminal of LipY possesses hormone-sensitive lipase homology and the conserved active-site motif GDSAG. LipY-deficient mutant was significantly impaired in triglycerol (TG) [Deb et al., 2006], suggestive of a role of LipY in the utilization of stored TG during dormancy and reactivation of *M. tuberculosis*. *M. marinum* $LipY_{mar}$ resembles LipY, with PE domain substituted by PPE domain. The triacylglycerol (TAG) pool is dramatically decreased in *M. smegmatis* overexpressing $LipY_{mar}$. A PPE domain deleted $LipY_{mar}$ yielded opposite result [Mishra et al., 2008], suggesting a shared role between PPE domains and PE domains. However, $LipY_{tub}$ and $LipY_{mar}$ cannot locate to cell wall in ESX-5 mutant [Daleke et al., 2011], implicating that LipYtub and LipYmar are both secreted into cell well through ESX-5 secretion system.

PPE PROTEINS AND ANTIGENIC VARIATION

Two mechanisms presumably underlie antigenic variation: the differential regulation and the structural genes mutation including point mutation, insertion, deletion, and frameshift mutations [Talarico et al., 2008]. Their highly polymorphic C-terminal domains [Cole et al., 1998; Cole, 1999] might contribute to the antigenic variation. *M. bovis* AF2122/97 and *M. tuberculosis* H37Rv genome comparison [Garnier et al., 2003] revealed that the sequence variation in 29 different PE_PGRS and 28 PPE proteins (belonging to the PPE_MPTR subfamily) originates from frameshifts, insertions, and deletions. The polymorphisms of MPTR domain of the PPE_ MPTR subfamily gene PPE8/Rv0355c can be found among more than 300 clinical isolates of *M. tuberculosis* [Srivastava et al., 2006]. This polymorphism holds true for PPE42/Rv2608 in clinical isolates of *M. tuberculosis* [Chakhaiyar et al., 2004]. However, further data are needed to corroborate the role of PPEs in antigenic variation, and do function in the host immune evasion or subversion.

PPE PROTEINS INTERFERE THE MACROPHAGE FUNCTIONALITIES

The maturation and acidification of phagosome are crucial macrophage defense mechanisms leading to the killing of intra-phagosomal pathogens. Some PE and PPE proteins may assist the *M. tuberculosis* persistence in host by subverting above function. *M. avium* PPE gene (64% homologous to *M. tuberculosis* PPE25/Rv1787) is up-regulated in macrophages [Li et al., 2005]. Transposon disruptants is deficient replication within macrophages, vacuole acidification, and endosome/phagosome fusion, implicating a role in the establishment of niche for bacterial macrophage intracellular survival [Li et al., 2005; Jha et al., 2010]. Four PE_PGRS mutants (PE_PGRS 5, 28, 44, 59) and three PPE_MPTR mutants (PPE_MPTR10, 16, 21) enriched in acidified phagosomes have been obtained from *M. bovis* BCG tranposon mutants library [Stewart et al., 2005]. Transposon-mediated *M. tuberculosis* (PPE54/Rv3343c) mutant was incapable of arresting phagosome maturation and rapid trafficking into acidified compartments [Brodin et al., 2010].

THE SIGNIFICANCE OF PPE FAMILY IN HOST IMMUNITY

PPE PROTEINS INVOLVE IN HUMORAL IMMUNITY

It is known that *Mycobacterium*-specific antibodies influence both *Mycobacterium* dissemination and inflammatory response, though the role of humoral immune responses in the control of *M. tuberculosis* infection is disputable. The highly repetitive domains of some PPE_MPTR subfamily members are presumably responsible for eliciting antibody responses. Previous study has shown that the Gly-X-Gly-Asn-X-Gly repeat motif of PPE41/Rv2430c can elicit both humoral immunity and cell-mediated immunity [Choudhary et al., 2003]. PPE42/Rv2608 showed positive reactivity to patients' serum samples through ELLSA and T cell-proliferation assays in patients with relapsed TB [Chakhaiyar et al., 2004]. Enzyme-linked immunosorbent assay (ELISA) has shown that PPE41/Rv2430c induced stronger B-cell response than other well-known antigens, such as Hsp10 or PPD [Choudhary et al., 2003]. In addition, PPE44/Rv2770c skewed the immune response towards a Th2 phenotype [Bonanni et al., 2005] as evidenced by the predominant of IgG1 isotype over IgG2a and the low IFN-γ and delayed-type hypersensitivity responses in BALB/c mice infected subcutaneously or intravenously with *M. bovis* BCG. The underlying molecular mechanic remains to be solved, especially how these PPEs bridge the humoral and cell mediated immunity.

PPE PROTEINS MODULATE CELL IMMUNITY

Functional CD8 T cells and CD4 T cell can initiate IFN-gamma production at different stage of *M. tuberculosis* infection in mice [Flynn et al., 1992; Caruso et al., 1999]. Therefore, cell-mediated immunity also plays an important role in the control of *M. tuberculosis* infection. T cell antigens may attribute to the development of subunit vaccine. C57BL/6 mice immunized with PPE14 Rv0915c DNA can develop both CD4-specific (predominantly Th1) and CD8-specific T cell responses to PPE14/Rv0915c protein [Skeiky et al., 2000]. PPE34/Rv1917c mediates the secretion of IL-4, IL-5, and IL-10 from CD4+ T cells [Bansal et al., 2010]. The CD8+ and CD4+ T cell populations and the splenocyte are significantly expanded in mouse which immunized with PPE41(Rv2430c) and PE25(Rv2431c)/PPE41(Rv2430c) protein complex as compared to PE25 (Tundup et al., 2008), suggesting that PPE proteins such as PPE41/Rv2430c may play an important role in T cell response. Furthermore, A potent T cell antigen Mtb39a encoded by PPE18 (Rv1196) elicited strongly the proliferation of T-cell and IFN-γ responses in peripheral blood mononuclear cells from 9 of 12 PPD-positive individuals tested [Dillon et al., 1999]. These indicate that PPE proteins can assist *Mycobacterium* evade host immune through cell-mediated immune and the identified T-cell epitopes might be ideal candidates for subunit vaccine against tuberculosis.

PPE PROTEINS REGULATE INNATE IMMUNITY

Proinflammatory cytokines are crucial for host defense against intracellular pathogen such as *M. tuberculosis*. Macrophage-derived IL-12 and TNF can influence the development of Th1-type T cell response and regulate the activation of immune response [Trinchieri, 2003]. In contrast, macrophage-induced IL-10 cytokine favors a Th2 T cell response [Nair et al., 2009]. Two well-documented PPE proteins were involved in MAPK and NF-κB signaling pathway via different mechanisms [Bansal et al., 2010; Nair et al., 2011]. PPE18/Rv1196c interacts specifically with TLR2, leading to sustained activation of p38 MAPK [Nair et al., 2009] and indirectly interacts with IkBa–NF-kB/rel complex through activation the phosphorylation of SOCS3 (suppressor of cytokine signaling 3) tyrosine residue, resulting in inhibition of NF-kB signaling [Nair et al., 2011]. Another PPE protein PPE34/Rv1917c can facilitate the subsequent immunity shift toward the Th2 phenotype by inducing the expression of cyclooxygenase-2 (COX-2), triggering the functional maturation of DCs and promote immune evasion of *Mycobacterium* through integration of cross-talk between PI3K-MAPK and NF-κB signaling cascades pathway [Bansal et al., 2010]. These suggest that some PPE proteins may effectively facilitate *M. tuberculosis* escaping host immune response through interfere with TLR-mediated signaling in innate immunity.

IMPLICATIONS FOR NEW ANTI-TB MEASURES

BIOMARKERS FOR TUBERCULOSIS SERODIAGNOSIS

Of the poor result of conventional diagnostic tests that based on purified protein derivative (PPD) necessitates better new diagnostic kits. The superior immunogenicity and sensitivity of some *M. tuberculosis* PPE family proteins highlighted their values [Singh et al., 2005; Khan et al., 2008; Wang et al., 2008]. PPE17/Rv1168c antigen can detect smear-negative pulmonary TB as well as extrapulmonary TB cases. Recombinant PPE17/Rv1168c protein displayed stronger immunoreactivity against the sera obtained from patients with clinically active TB than PPD, Hsp60, or ESAT-6 and could distinguish TB patients from *M. bovis* BCG-vaccinated controls as well [Khan et al., 2008]. The IgG response to recombinant PPE57/Rv3425 was nearly equal to the well-known antigen CFP-10 and higher than ESAT-6 [Zhang et al., 2007]. Antibodies to the C terminal of PPE55/Rv3347c can be detected during almost all infection stages, such as the retrospective sera obtained months

prior to manifestation of clinical TB from HIV(+) TB(+) individuals, subclinical TB in HIV-infected humans but absent in the sera of positive healthy controls [Singh et al., 2005]. In addition, PPE44/Rv2770c-specific immune responses are detected in mice acutely, chronically, and latently infected with *M. tuberculosis* as well. Together, these may suggest that some PPE proteins such as PPE57/Rv3425, PPE55/Rv3347c, PPE17/Rv1168c, and PPE44/Rv2770c may be the potential biomarkers to detect latent, incipient and subclinical tuberculosis [Romano et al., 2008]. A more rational combination of these potential biomarkers for tuberculosis serodiagnosis might be more sensitive, specific, and reliable. A multicenter evaluation of these combinations are needed to valid the huge potential.

POTENTIAL SUBUNIT VACCINE COMPONENTS

PPE proteins might be ideal vaccine candidates, several PPEs have been assessed for possible inclusion in new vaccines [Bertholet et al., 2008; Romano et al., 2008; Wang et al., 2009]. Some PPE proteins have demonstrated great promise as vaccine components including adjuvants (PPE42/Rv2608 and PPE44/Rv2770c) and T-cell antigen (PPE57/Rv3425 and PPE68 /Rv3873). PPE42 (Rv2608) was shown to confer partial protection in mice when formulated with the TLR-9 agonist CpG [Bertholet et al., 2008]. The fusion protein ID83 (a mixture of Rv1813, Rv3620, and PPE42/Rv2608) elicits protective immunity in mice [Bertholet et al., 2008], with efficacy varies with adjuvants and route of immunization [Baldwin et al., 2009]. Recombinant PPE44 (Rv2770c) protein formulated in adjuvant generated strong cellular and humoral immune responses comparable to BCG [Romano et al., 2008], indicating that PPE44/Rv2770c of *M. tuberculosis* is a protective antigen that might be included in novel subunit TB vaccines [Cuccu et al., 2011]. PPE68/Rv3873, like ESAT-6 and CFP10, is also a potent T-cell antigen recognized by *M. tuberculosis*-infected individuals [Okkels et al., 2003]. Both recombinant BCG co-expression PPE57/Rv3425 and Ag85B (rBCG::Ag85B-Rv3425) elicit stronger IFN-γ in antigen-stimulated T cells and increased specific IgG titers in C57BL/6 mice [Wang et al., 2009].

Development of effective TB control strategies remains one of the most formidable challenges. PPE proteins play important role in the host–pathogen interactions. More details on PPEs can better serve future TB control measures development.

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