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Mycobacterium tuberculosis RD-1 Secreted Antigens as Protective and Risk Factors for Tuberculosis

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1. Introduction

Mycobacterium tuberculosis (Mtb) infects about 8 million people every year and causes death of about 2-3 million (Raviglione, 2003). In recent times, there has been a wider spread of tuberculosis, mainly due to emergence of multi drug resistance (MDR) bacilli and enhanced susceptibility to the disease by patients infected with human immunodeficiency virus (HIV) (Elliot et al., 1995; Chintu and Mwinga, 1999). Transmission of the infection by Mtb bacilli is air borne and occurs through inhalation of aerosol containing the bacilli exhaled by coughing, sneezing or spitting by patients suffering from pulmonary tuberculosis. The inhaled bacilli are engulfed by the alveolar macrophages, where the bacilli are able to persist successfully in a latent or proliferating state. This persistence is achieved by modulation of several intracellular signaling pathways in order to create a suitable environment for the bacilli. The interplay of mycobacteria with host signaling pathways is a complex and dynamic process that is not clearly understood. Mtb secretes several molecules that modulate the signaling pathways (Koul et al., 2004). Most of these molecules commonly target macrophages, which helps the bacilli to evade innate immune response and propagate throughout the system (Rosenberger and Finlay, 2003).

The proteins secreted by Mtb have gained attention in recent years as putative vaccine and diagnostic candidates (Harboe et al., 1996; Colangeli et al., 2000). But there have been recent reports about their role in modulation of macrophage signaling pathways leading to compromise of macrophage functions (Trajkovic et al., 2002; Pym et al., 2003; Guinn et al., 2004). Thus the secretory proteins can act as risk or virulent factors too. This notion is also supported by the fact that only live but not dead bacilli can down regulate macrophage functions (Malik et al., 2001). In this chapter, we focus our discussion on the role of the

proteins secreted by region of difference-1 (RD-1), the region that is deleted in all the strains of *M. bovis* BCG.

2. Genetic architecture of Region of Difference-1 (RD-1)

Comparative genome analysis using DNA microarray, bacterial artificial chromosomes (BAC) and the subtractive hybridization between virulent and attenuated strains of Mtb complex and *M. bovis* BCG identified several regions of difference (RD) (Behr et al., 1999; Gordon et al., 1999; Mahairas et al., 1996). A gene segment of 9.5kb that encompasses nine open reading frames (ORF) of Rv3871-Rv3879c is present in virulent strains of Mtb, and which is deleted consistently in all the strains of *M. bovis* BCG (Cole et al., 1998). This region was designated as RD-1. Two of these ORFs, Rv3874 and Rv3875, encode 10-kDa culture filtrate protein (CFP-10) and 6-kDa early secreted antigenic target (ESAT-6) protein respectively. Interestingly, deletion of the RD-1 fragments from Mtb causes loss of its virulence, while introduction of the RD-1 locus into *M. bovis* BCG or *M. microti* resulted in increased virulence and survival properties (Behr, 2002; Pym et al., 2002; Lewis et al., 2003; Demangel et al., 2005). This review will focus on the role of these two proteins in modulation of the macrophage signaling pathways and macrophage functions for the bacteria to persist for longer time. We also discuss about the potential role of these proteins as vaccine candidates owing to their high immunogenicity.

3. Structural biology of CFP-10 and ESAT-6

The ORFs Rv3874 and Rv3875 encoding CFP10 and ESAT6 respectively are cotranscribed into a single RNA product (Cole et al., 1998). Nuclear magnetic resonance (NMR) spectroscopy showed that CFP10 exhibits very little secondary structure and consists mostly of random coils, which are unstructured. The ESAT6, on the other hand has 75% secondary structure in the form of α-helices (Renshaw et al., 2002). One interesting study showed that CFP10 and ESAT6 forms a tight 1:1 complex where both proteins adopt more stable and folded configuration than the native moieties (Renshaw et al., 2002; Lightbody et al., 2004). The complex formation between the two proteins is hydrophobic in nature and led to a significant increase in the helical content of the two proteins. Inside the core of the complex, helix-turn-helix motifs of the two proteins form a quad-helix bundle (Renshaw et al., 2005). Within the complex, the flexible C-terminus of CFP-10 is involved in binding to the cell surface. This was confirmed by the fact that deletion 87 amino acids at C-terminus of CFP-10 inhibited the binding of complex to the cell surface, while deletion of the same in ESAT-6 had no effect (Renshaw et al., 2005). The complex formation between the two proteins was reversible and the complex broke down to individual proteins at 53.4^0C. The complex was also shown to be more stable to proteolytic digestion by trypsin (Meher at al., 2006). The enhanced stability to proteolytic digestion caused lower T cell activation compared to ESAT-6 alone (Marei et al., 2005). ESAT-6 protein was also found to have auto-proteolytic activity; it can self cleave-off six amino acids at the C-terminus which are responsible for its binding to the cell surface. A mutant ESAT-6 lacking these six amino acids was unable to bind to cell surface (Pathak et al., 2007).

4. Use of CFP10 and ESAT6 as tools for diagnosis and vaccine

CFP-10 and ESAT-6 were identified in a screen to identify the proteins present in culture filtrates of Mtb and *M. bovis BCG*, which could induce T cell mediated response. (Andersen et al., 1991a; Andersen et al., 1991b; Andersen et al., 1994; Weldingh et al., 1998; Weldingh et al., 1999). The screen yielded six low molecular weight antigens *viz*. Rv3871, Rv3872, Rv3873, CFP-10, ESAT-6 and Rv3878. These antigens when expressed and purified as recombinant proteins gave strong humoral response in tuberculous guinea pigs while only two antigens i.e. CFP-10 and ESAT-6 showed strong delayed type hypersensitivity (DTH) reaction in the guinea pigs (Weldingh et al., 1999). CFP10 and ESAT6 are potent T cell antigens and induce strong T cell response. In mice infected with Mtb, CFP-10 specific T cells were observed at quite early stage of infection in lungs. These T cells were activated by CFP-10 epitopes and were recruited in large numbers (Kamath et al., 2004). This resulted in production of large amounts of interferon-γ (IFN-γ). Recombinant CFP-10 has also been shown to be a potent T cell antigen, inducing T cell proliferation and IFN-γ production in peripheral blood mononuclear cells in about 70% of purified protein derivative (PPD) positive asymptomatic individuals. CFP-10 was also shown to induce delayed type hypersensitivity (DTH) in Mtb infected guinea pigs but not in *M. bovis* BCG infected guinea pigs (Colangeli et al., 2000). ESAT-6 is also a RD-1 antigen inducing robust levels of IFN-γ by T cells in early stages of *M. tuberculosis* infection (Porsa et al., 2006; Ravn et al., 1999; Skjot et al., 2000; de Jong et al., 2006). Two different T cell epitopes were observed in mice, which were recognized by different MHCII molecules under different circumstances (Dietrich et al., 2005).

5. Macrophage subversion by CFP10 and ESAT6

Despite their well-known role as T cell antigens, CFP10 and ESAT6 modulate several pathways inside the macrophage, thereby creating a suitable environment to persist inside the host cell. Studies from our lab have shown that CFP-10 and ESAT-6 downregulates the production of reactive oxygen species (ROS) inside the macrophages; which in turn dampens the NF-κB transactivation property (Ganguly et al., 2008a, Ganguly et al., 2008b). The inhibition of ROS production was greater with the CFP10:ESAT6 complex compared to the individual proteins. Most of the effects of these proteins seem to be mediated by Toll-like receptors (TLR). Analysis of global phospho-proteome in CFP-10 treated J774.1 macrophages showed that CFP-10 caused de-phosphorylation of a large number of macrophage proteins (Basu et al., 2006; Basu et al., 2009). The de-phsophorylation occurs due to increase in activity of membrane tyrosine phosphatases SHP-1 and SHP-2 (Src homology domain proteins). The increased phosphatase activity is due to reduction in production of ROS inside the macrophages. The ROS production in macrophages occurs through NADPH oxidase pathway. These observations suggest that upon binding of CFP-10 and ESAT-6 to macrophage surface, Mtb is able to reduce the burst of ROS inside the cell which contributes to bactericidal activity. Thus it might be one of the survival strategies of the bacilli. Mtb contains several enzymes to deal with the ROS/oxidative burst like catalase, peroxidase (Kat) (Sherman et

al., 1995; Manca et al., 1999; Ng et al., 2004) as well as superoxide dismutases Sod A and Sod C (Piddington et al., 2001; Zhang et al., 1991). ESAT-6 was also found to inhibit mitogen activated kinase/extracellular signal regulated kinases 1/2 (MAPK/ERK1/2). This occurs due to some phosphatase activity in the nucleus which dephosphorylates ERK1/2. This resulted in reduction in lipopolysaccharide (LPS) induced expression of transcription factor c-myc (Ganguly et al., 2007). ESAT-6 also reduced the LPS-induced expression of several genes like *IL-1β*, *Bax*, *Icam-1* and *tnfr-1*. Recent studies have shown that ESAT-6 binds to toll-like receptor-2 (TLR2) on the macrophage surface; and the six amino acids at the C-terminus of the protein are critical for its TLR2 binding (Pathak et al., 2007). This binding caused inactivation of transcription factors interferon regulatory factors (IRF) and NF-κB. Recent observations from our lab have shown that ESAT-6 down-regulates IFN-γ inducible expression of type I and type IV isomers of MHC class II transactivator (CIITA) in macrophages (Kumar et al., 2011). Interestingly, the downregulation of type I CIITA was independent of TLR-2 while the effect on type IV CIITA was mediated through TLR-2. This suggests that ESAT-6 may bind to other TLRs or some other receptor on macrophages. Another study has shown that ESAT-6 was able to induce apoptosis in human monocytic cell line THP-1 through activation of caspases (Derrick et al., 2007). It was also shown that ESAT-6 could induce pore formation on the surface of some cell types.

Apart from macrophages, CFP-10 and ESAT-6 also modulate functions in dendritic cells (Natarajan et al., 2011; Trajkovic et al., 2004). Studies have shown that CFP-10 induced differentiation of bone marrow cells into dendritic cells (DC) and this involved activation of NF-κB (Latchumanan et al., 2002). CFP-10 also induced maturation of DCs, which caused downregulation of pro-inflammatory cytokines like interleukin-2 (IL-2) and IFN-γ (Natarajan et al., 2003). The CFP-10-differentiated and CFP-10-matured DCs when cultured with the Mtb whole-cell-extract primed T cells, showed reduced levels of pro-inflammatory cytokines IL-12p40 and IFN-γ along with elevated levels of anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF-β) (Balkhi et al., 2004). Therefore CFP-10 primes DCs to have reduced efficacy to eliminate Mtb. CFP-10 also reduced ROS production during differentiation of DCs compared to the positive stimulator granulocyte macrophage colony-an stimulating factor (GM-CSF). This downregulation of ROS resulted in increased survival of *M. bovis* BCG in these DCs (Sinha et al., 2006). The CFP-10 differentiated DCs also had reduced levels of chemokines RANTES and IP-10 upon infection by mycobacteria (Salam et al., 2008).

6. Virulence linked to CFP10/ESAT6 secretion

CFP-10 and ESAT-6 proteins are secreted without any secretory signal sequence outside the host cell. The secretory apparatus that helps in the secretion of these two proteins have been characterized recently (Guinn et al., 2004; Fortune et al., 2005; Brodin et al., 2006). The CFP10/ESAT6 secretion system is an active process involving several genes, some of them being ATP hydrolyzing chaperones (Stanley et al., 2003; Converse and Cox, 2005; Stanley et al., 2007). Mutations in these genes caused significant reduction in virulence of Mtb in mouse model (Stanley et al., 2003; Majlessi et al., 2005). This

attenuation could be due to the inhibition of CFP10/ESAT6 secretion, which further highlights the role of these two proteins in modulation of macrophage function. A study has shown that one of the components of ESX-1 secretion system, Rv3871 binds to the C-terminus of CFP-10, and this facilitates the secretion of both CFP-10 and ESAT-6. Mutations at the C-terminus resulted in loss of binding to CFP-10 and impaired secretion of the two proteins (Champion et al., 2006). This suggests that CFP-10 and ESAT-6 might be secreted in the form of heterodimeric 1:1 complex out of the cell. The ESX-1 system has four paralogues in Mtb and some of them have been shown to be essential for in-vitro growth of the bacilli (Simeone et al., 2009). In the ESX-1 system, Rv3868, Rv3869, Rv3870, Rv3871 and Rv3877 have been shown to be essential for CFP-10/ESAT-6 secretion while loss of Rv3865 and partial loss of Rv3866 did not affect protein secretion, rather it caused attenuation of the bacilli (Brodin et al., 2006). Thus Rv3865/3866 might be some virulence factor that does not control ESAT-6 secretion. Studies with mutant bacilli showed that ESX-1 system is required for the induction of type I IFN induction that in turn contributes to the spread of the bacilli (Stanley et al., 2007). In *Mycobacterium marinum*, the CFP-10/ESAT-6 secretion manipulates the phagosome-lysosome fusion. Mutations in this secretion system results in enhanced phagosome-lysosome fusion and reduced survival of mycobacteria (Tan et al., 2006; Majlessi et al., 2005; Champion et al., 2006; Xu et al., 2007; Lee et al., 2001). Analyses of deletion mutants of ESAT-6 have identified the key amino acids in complex formation, virulence and secretion (Brodin et al., 2005). The Trp-X-Gly motif on ESAT-6 is involved in complex formation with CFP-10, virulence and induction of specific T cell responses whereas mutations in the six amino acids at the C-terminus had no effect on secretion but caused attenuation. At acidic pH (as normally found in phagosomes), ESAT-6 dissociated from its complexing partner CFP-10 and bound to liposomes, which caused lysis of the liposomes (de Jonge et al., 2007). This could be a mechanism for the Mtb to escape degradation within the phagosome. In dendritic cells, Mtb translocated from phagolysosome to cytoplasm, which is dependent upon the CFP-10/ESAT-6 secretion (van der Wel et al., 2007). This translocation resulted in the death of the host cell.

7. Conclusions

The interaction between mycobacteria and the host macrophage or dendritic cells is very complex and dependent on multiple factors. In this review, we have focused mainly on the modulating activities of CFP10/ESAT6, the molecules which are being evaluated as vaccine candidates, indicating that they may act like double-edged sword generating a favorable response from the host immune system. Apart from CFP-10/ESAT-6, several other protein antigens are also being reported which modulate the macrophage response to Mtb. Further studies are undergoing in our lab to elucidate the finer mechanisms by which these proteins function.

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9. References

Andersen, P. (1994). Effective vaccination of mice against *Mycobacterium tuberculosis* infection with a soluble mixture of secreted mycobacterial proteins. Infection and Immunity 62, 2536-2544.

Andersen, P., Askgaard, D., Ljungqvist, L., Bennedsen, J. & Heron, I. (1991a). Proteins released from *Mycobacterium tuberculosis* during growth. Infection and Immunity 59, 1905-1910.

Andersen, P., Askgaard, D., Ljungqvist, L., Bentzon, M.W. & Heron, I. (1991b). T-cell proliferative response to antigens secreted by *Mycobacterium tuberculosis*. Infection and Immunity 59, 1558-1563.

Balkhi, M.Y., Sinha, A. & Natarajan, K. (2004). Dominance of CD86, transforming growth factor- beta 1, and interleukin-10 in *Mycobacterium tuberculosis* secretory antigen-activated dendritic cells regulates T helper 1 responses to mycobacterial antigens. The Journal of Infectious Diseases 189, 1598-1609.

Basu, S.K., Kumar, D., Singh, D.K., Ganguly, N., Siddiqui, Z., Rao, K.V. & Sharma, P. (2006). *Mycobacterium tuberculosis* secreted antigen (MTSA-10) modulates macrophage function by redox regulation of phosphatases. The FEBS Journal 273, 5517-5534.

Behr, M.A. (2002). BCG--different strains, different vaccines? The Lancet Infectious Diseases 2, 86-92.

Behr, M.A., Wilson, M.A., Gill, W.P., Salamon, H., Schoolnik, G.K., Rane, S. & Small, P.M. (1999). Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science 284, 1520-1523.

Berthet, F.X., Rasmussen, P.B., Rosenkrands, I., Andersen, P. & Gicquel, B. (1998). A *Mycobacterium tuberculosis* operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10). Microbiology 144 (Pt 11), 3195-3203.

Brodin, P., de Jonge, M.I., Majlessi, L., Leclerc, C., Nilges, M., Cole, S.T. & Brosch, R. (2005). Functional analysis of early secreted antigenic target-6, the dominant T-cell antigen of *Mycobacterium tuberculosis*, reveals key residues involved in secretion, complex formation, virulence, and immunogenicity. The Journal of Biological Chemistry 280, 33953-33959.

Brodin, P., Majlessi, L., Marsollier, L., de Jonge, M.I., Bottai, D., Demangel, C., Hinds, J., Neyrolles, O., Butcher, P.D., Leclerc, C., Cole, S.T. & Brosch, R. (2006). Dissection of ESAT-6 system 1 of *Mycobacterium tuberculosis* and impact on immunogenicity and virulence. Infection and Immunity 74, 88-98.

Brodin, P., Rosenkrands, I., Andersen, P., Cole, S.T. & Brosch, R. (2004). ESAT-6 proteins: protective antigens and virulence factors? Trends in Microbiology 12, 500-508.

Champion, P.A., Stanley, S.A., Champion, M.M., Brown, E.J. & Cox, J.S. (2006). C-terminal signal sequence promotes virulence factor secretion in *Mycobacterium tuberculosis*. Science 313, 1632-1636.

Chintu, C. & Mwinga, A. (1999). An African perspective on the threat of tuberculosis and HIV/AIDS--can despair be turned to hope? Lancet 353, 997.

Colangeli, R., Spencer, J.S., Bifani, P., Williams, A., Lyashchenko, K., Keen, M.A., Hill, P.J., Belisle, J. & Gennaro, M.L. (2000). MTSA-10, the product of the Rv3874 gene of

Mycobacterium tuberculosis, elicits tuberculosis-specific, delayed-type hypersensitivity in guinea pigs. Infection and Immunity 68, 990-993.

Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., Eiglmeier, K., Gas, S., Barry, C.E., 3rd, Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gentles, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., McLean, J., Moule, S., Murphy, L., Oliver, K., Osborne, J., Quail, M.A., Rajandream, M.A., Rogers, J., Rutter, S., Seeger, K., Skelton, J., Squares, R., Squares, S., Sulston, J.E., Taylor, K., Whitehead, S. & Barrell, B.G. (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393, 537-544.

Converse, S.E. & Cox, J.S. (2005). A protein secretion pathway critical for *Mycobacterium tuberculosis* virulence is conserved and functional in *Mycobacterium smegmatis*. Journal of Bacteriology 187, 1238-1245.

de Jong, B.C., Hill, P.C., Brookes, R.H., Gagneux, S., Jeffries, D.J., Otu, J.K., Donkor, S.A., Fox, A., McAdam, K.P., Small, P.M. & Adegbola, R.A. (2006). *Mycobacterium africanum* elicits an attenuated T cell response to early secreted antigenic target, 6 kDa, in patients with tuberculosis and their household contacts. The Journal of Infectious Diseases 193, 1279-1286.

de Jonge, M.I., Pehau-Arnaudet, G., Fretz, M.M., Romain, F., Bottai, D., Brodin, P., Honore, N., Marchal, G., Jiskoot, W., England, P., Cole, S.T. & Brosch, R. (2007). ESAT-6 from *Mycobacterium tuberculosis* dissociates from its putative chaperone CFP-10 under acidic conditions and exhibits membrane-lysing activity. Journal of Bacteriology 189, 6028-6034.

Demangel, C., Garnier, T., Rosenkrands, I. & Cole, S.T. (2005). Differential effects of prior exposure to environmental mycobacteria on vaccination with *Mycobacterium bovis* BCG or a recombinant BCG strain expressing RD1 antigens. Infection and Immunity 73, 2190-2196.

Derrick, S.C. & Morris, S.L. (2007). The ESAT6 protein of *Mycobacterium tuberculosis* induces apoptosis of macrophages by activating caspase expression. Cellular Microbiology 9, 1547-1555.

Dietrich, J., Aagaard, C., Leah, R., Olsen, A.W., Stryhn, A., Doherty, T.M. & Andersen, P. (2005). Exchanging ESAT6 with TB10.4 in an Ag85B fusion molecule-based tuberculosis subunit vaccine: efficient protection and ESAT6-based sensitive monitoring of vaccine efficacy. Journal of Immunology 174, 6332-6339.

Elliot, A.M., Halwindii, B., Hayes, R., Luo, N., Mwinga, A.G., Tembo, G., Machiels, L., Steenbergen, G., Pobee, J.O., Nunn, P.P., et al. (1995). The impact of human immunodeficiency virus on response to treatment and recurrence rate in patients treated for tuberculosis: two-year follow-up of a cohort in Lusaka, Zambia. J Trop Med Hyg. 98, 9-21.

Fortune, S.M., Jaeger, A., Sarracino, D.A., Chase, M.R., Sassetti, C.M.,Sherman, D.R., Bloom, B.R. & Rubin, E.J. (2005). Mutually dependent secretion of proteins required for mycobacterial virulence. Proceedings of the National Academy of Sciences of the United States of America 102, 10676-10681.

Ganguly, N., Giang, P.H., Basu, S.K., Mir, F.A., Siddiqui, I. & Sharma, P. (2007). *Mycobacterium tuberculosis* 6-kDa early secreted antigenic target (ESAT-6) protein downregulates lipopolysaccharide induced c-myc expression by modulating the extracellular signal regulated kinases 1/2. BMC Immunology 8, 24.

Ganguly, N., Giang, P.H., Gupta, C., Basu, S.K., Siddiqui, I., Salunke, D.M. & Sharma, P. (2008a). *Mycobacterium tuberculosis* secretory proteins CFP-10, ESAT-6 and the CFP10:ESAT6 complex inhibit lipopolysaccharide-induced NF-kappaB transactivation by downregulation of reactive oxidative species (ROS) production. Immunology and Cell Biology 86, 98-106.

Ganguly, N., Siddiqui, I. & Sharma, P. (2008b). Role of *M. tuberculosis* RD-1 region encoded secretory proteins in protective response and virulence. Tuberculosis 88, 510-517.

Gordon, S.V., Brosch, R., Billault, A., Garnier, T., Eiglmeier, K. & Cole, S.T. (1999). Identification of variable regions in the genomes of tubercle bacilli using bacterial artificial chromosome arrays. Molecular Microbiology 32, 643-655.

Guinn, K.M., Hickey, M.J., Mathur, S.K., Zakel, K.L., Grotzke, J.E., Lewinsohn, D.M., Smith, S. & Sherman, D.R. (2004). Individual RD1-region genes are required for export of ESAT-6/CFP-10 and for virulence of *Mycobacterium tuberculosis*. Molecular Microbiology 51, 359-370.

Harboe, M., Oettinger, T., Wiker, H.G., Rosenkrands, I. & Andersen, P. (1996). Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. Infection and Immunity 64, 16-22.

Kamath, A.B., Woodworth, J., Xiong, X., Taylor, C., Weng, Y. & Behar, S.M. (2004). Cytolytic CD8+ T cells recognizing CFP10 are recruited to the lung after *Mycobacterium tuberculosis* infection. The Journal of Experimental Medicine 200, 1479-1489.

Khan, I.H., Ravindran, R., Yee, J., Ziman, M., Lewinsohn, D.M., Gennaro, M.L., Flynn, J.L., Goulding, C.W., DeRiemer, K., Lerche, N.W. & Luciw, P.A. (2008). Profiling antibodies to *Mycobacterium tuberculosis* by multiplex microbead suspension arrays for serodiagnosis of tuberculosis. Clinical and Vaccine Immunology 15, 433-438.

Koul, A., Herget, T., Klebl, B.& Ullrich, A. (2004). Interplay between mycobacteria and host signalling pathways. Nature reviews. Microbiology 2, 189-202.

Kumar, P., Agarwal, R., Siddiqui, I., Vora, H., Das, G. & Sharma, P. (2011). ESAT6 differentially inhibits IFN-gamma-inducible class II transactivator isoforms in both a TLR2-dependent and -independent manner. Immunology and Cell Biology advance online publication 14 June 2011; doi: 10.1038/icb.2011.54

Latchumanan, V.K., Balkhi, M.Y., Sinha, A., Singh, B., Sharma, P. & Natarajan, K. (2005). Regulation of immune responses to *Mycobacterium tuberculosis* secretory antigens by dendritic cells. Tuberculosis 85, 377-383.

Latchumanan, V.K., Singh, B., Sharma, P. & Natarajan, K. (2002). *Mycobacterium tuberculosis* antigens induce the differentiation of dendritic cells from bone marrow. Journal of Immunology 169, 6856-6864.

Lee, V.T. & Schneewind, O. (2001). Protein secretion and the pathogenesis of bacterial infections. Genes & Development 15, 1725-1752.

Lewis, K.N., Liao, R., Guinn, K.M., Hickey, M.J., Smith, S., Behr, M.A. & Sherman, D.R. (2003). Deletion of RD1 from *Mycobacterium tuberculosis* mimics bacille Calmette-Guerin attenuation. The Journal of Infectious Diseases 187, 117-123.

Lightbody, K.L., Renshaw, P.S., Collins, M.L., Wright, R.L., Hunt, D.M., Gordon, S.V., Hewinson, R.G., Buxton, R.S., Williamson, R.A. & Carr, M.D. (2004). Characterisation of complex formation between members of the *Mycobacterium tuberculosis* complex CFP-10/ESAT-6 protein family: towards an understanding of the rules governing complex formation and thereby functional flexibility. FEMS Microbiology Letters 238, 255-262.

Mahairas, G.G., Sabo, P.J., Hickey, M.J., Singh, D.C. & Stover, C.K. (1996). Molecular analysis of genetic differences between *Mycobacterium bovis* BCG and virulent M. bovis. The Journal of Bacteriology 178, 1274-1282.

Majlessi, L., Brodin, P., Brosch, R., Rojas, M.J., Khun, H., Huerre, M., Cole, S.T. & Leclerc, C. (2005). Influence of ESAT-6 secretion system 1 (RD1) of *Mycobacterium tuberculosis* on the interaction between mycobacteria and the host immune system. Journal of Immunology 174, 3570-3579.

Malik, Z.A., Iyer, S.S. & Kusner, D.J. (2001). *Mycobacterium tuberculosis* phagosomes exhibit altered calmodulin-dependent signal transduction: contribution to inhibition of phagosome-lysosome fusion and intracellular survival in human macrophages. Journal of Immunology 166, 3392-3401.

Manca, C., Paul, S., Barry, C.E., 3rd, Freedman, V.H.& Kaplan, G. (1999). *Mycobacterium tuberculosis* catalase and peroxidase activities and resistance to oxidative killing in human monocytes in vitro. Infection and Immunity 67, 74-79.

Marei, A., Ghaemmaghami, A., Renshaw, P., Wiselka, M., Barer, M., Carr, M. & Ziegler-Heitbrock, L. (2005). Superior T cell activation by ESAT-6 as compared with the ESAT-6-CFP-10 complex. International Immunology 17, 1439-1446.

Meher, A.K., Bal, N.C., Chary, K.V. & Arora, A. (2006). *Mycobacterium tuberculosis* H37Rv ESAT-6-CFP-10 complex formation confers thermodynamic and biochemical stability. The FEBS Journal 273, 1445-1462.

Natarajan, K., Kundu, M., Sharma, P. & Basu, J. (2011). Innate immune responses to *M. tuberculosis* infection. Tuberculosis (2011), doi:10.1016/j.tube.2011.04.003.

Natarajan, K., Latchumanan, V.K., Singh, B., Singh, S. & Sharma, P. (2003). Down-regulation of T helper 1 responses to mycobacterial antigens due to maturation of dendritic cells by 10-kDa mycobacterium tuberculosis secretory antigen. The Journal of Infectious Diseases 187, 914-928.

Ng, V.H., Cox, J.S., Sousa, A.O., MacMicking, J.D. & McKinney, J.D. (2004). Role of KatG catalase-peroxidase in mycobacterial pathogenesis: countering the phagocyte oxidative burst. Molecular Microbiology 52, 1291-1302.

Pathak, S.K., Basu, S., Basu, K.K., Banerjee, A., Pathak, S., Bhattacharyya, A., Kaisho, T., Kundu, M. & Basu, J. (2007). Direct extracellular interaction between the early secreted antigen ESAT-6 of *Mycobacterium tuberculosis* and TLR2 inhibits TLR signaling in macrophages. Nature Immunology 8, 610-618.

Piddington, D.L., Fang, F.C., Laessig, T., Cooper, A.M., Orme, I.M. & Buchmeier, N.A. (2001). Cu,Zn superoxide dismutase of *Mycobacterium tuberculosis* contributes to survival in activated macrophages that are generating an oxidative burst. Infection and Immunity 69, 4980-4987.

Porsa, E., Cheng, L., Seale, M.M., Delclos, G.L., Ma, X., Reich, R., Musser, J.M. & Graviss, E.A. (2006). Comparison of a new ESAT-6/CFP-10 peptide-based gamma interferon assay and a tuberculin skin test for tuberculosis screening in a moderate-risk population. Clinical and Vaccine Immunology 13, 53-58.

Pym, A.S., Brodin, P., Brosch, R., Huerre, M. & Cole, S.T. (2002). Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines *Mycobacterium bovis* BCG and *Mycobacterium microti*. Molecular Microbiology 46, 709-717.

Pym, A.S., Brodin, P., Majlessi, L., Brosch, R., Demangel, C., Williams, A., Griffiths, K.E., Marchal, G., Leclerc, C. & Cole, S.T. (2003). Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis. Nature Medicine 9, 533-539.

Raviglione, M.C. (2003). The TB epidemic from 1992 to 2002. Tuberculosis 83, 4-14.

Ravn, P., Demissie, A., Eguale, T., Wondwosson, H., Lein, D., Amoudy, H.A., Mustafa, A.S., Jensen, A.K., Holm, A., Rosenkrands, I., Oftung, F., Olobo, J., von Reyn, F. & Andersen, P. (1999). Human T cell responses to the ESAT-6 antigen from *Mycobacterium tuberculosis*. The Journal of Infectious Diseases 179, 637-645.

Renshaw, P.S., Lightbody, K.L., Veverka, V., Muskett, F.W., Kelly, G., Frenkiel, T.A., Gordon, S.V., Hewinson, R.G., Burke, B., Norman, J., Williamson, R.A. & Carr, M.D. (2005). Structure and function of the complex formed by the tuberculosis virulence factors CFP-10 and ESAT-6. The EMBO Journal 24, 2491-2498.

Renshaw, P.S., Panagiotidou, P., Whelan, A., Gordon, S.V., Hewinson, R.G., Williamson, R.A. & Carr, M.D. (2002). Conclusive evidence that the major T-cell antigens of the *Mycobacterium tuberculosis* complex ESAT-6 and CFP-10 form a tight, 1:1 complex and characterization of the structural properties of ESAT-6, CFP-10, and the ESAT-6*CFP-10 complex. Implications for pathogenesis and virulence. The Journal of Biological Chemistry 277, 21598-21603.

Rosenberger, C.M. & Finlay, B.B. (2003). Phagocyte sabotage: disruption of macrophage signalling by bacterial pathogens. Nature reviews. Molecular Cell Biology 4, 385-396.

Salam, N., Gupta, S., Sharma, S., Pahujani, S., Sinha, A., Saxena, R.K. & Natarajan, K. (2008). Protective immunity to *Mycobacterium tuberculosis* infection by chemokine and cytokine conditioned CFP-10 differentiated dendritic cells. PloS One 3, e2869.

Sherman, D.R., Sabo, P.J., Hickey, M.J., Arain, T.M., Mahairas, G.G., Yuan, Y., Barry, C.E., 3rd, & Stover, C.K. (1995). Disparate responses to oxidative stress in saprophytic and pathogenic mycobacteria. Proceedings of the National Academy of Sciences of the United States of America 92, 6625-6629.

Simeone, R., Bottai, D. & Brosch, R. (2009). ESX/type VII secretion systems and their role in host-pathogen interaction. Current Opinion in Microbiology 12, 4-10.

Singh, A., Mai, D., Kumar, A. & Steyn, A.J. (2006). Dissecting virulence pathways of *Mycobacterium tuberculosis* through protein-protein association. Proceedings of the National Academy of Sciences of the United States of America 103, 11346-11351.

Sinha, A., Singh, A., Satchidanandam, V. & Natarajan, K. (2006). Impaired generation of reactive oxygen species during differentiation of dendritic cells (DCs) by *Mycobacterium tuberculosis* secretory antigen (MTSA) and subsequent activation of MTSA-DCs by mycobacteria results in increased intracellular survival. Journal of Immunology 177, 468-478.

Skjot, R.L., Oettinger, T., Rosenkrands, I., Ravn, P., Brock, I., Jacobsen, S. & Andersen, P. (2000). Comparative evaluation of low-molecular-mass proteins from *Mycobacterium tuberculosis* identifies members of the ESAT-6 family as immunodominant T-cell antigens. Infection and Immunity 68, 214-220.

Stanley, S.A., Johndrow, J.E., Manzanillo, P. & Cox, J.S. (2007). The Type I IFN response to infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion and contributes to pathogenesis. Journal of Immunology 178, 3143-3152.

Stanley, S.A., Raghavan, S., Hwang, W.W. & Cox, J.S. (2003). Acute infection and macrophage subversion by *Mycobacterium tuberculosis* require a specialized secretion system. Proceedings of the National Academy of Sciences of the United States of America 100, 13001-13006.

Tan, T., Lee, W.L., Alexander, D.C., Grinstein, S. & Liu, J. (2006). The ESAT-6/CFP-10 secretion system of *Mycobacterium marinum* modulates phagosome maturation. Cellular Microbiology 8, 1417-1429.

Trajkovic, V., Natarajan, K. & Sharma, P. (2004). Immunomodulatory action of mycobacterial secretory proteins. Microbes and Infection 6, 513-519.

Trajkovic, V., Singh, G., Singh, B., Singh, S., and Sharma, P. (2002). Effect of Mycobacterium tuberculosis-specific 10-kiloDaltn antigen on macrophage release of tumour necrosis factor-alpha and nitric oxide. Infection & Immunity 70, 6558-6566.

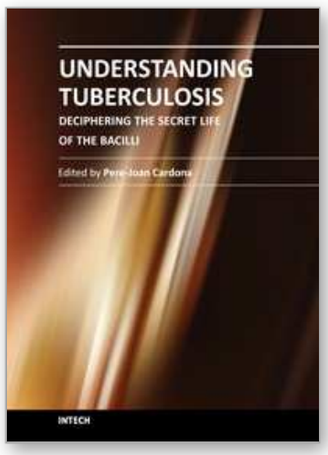
van der Wel, N., Hava, D., Houben, D., Fluitsma, D., van Zon, M., Pierson, J., Brenner, M. & Peters, P.J. (2007). *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. Cell 129, 1287-1298.

Weldingh, K. & Andersen, P. (1999). Immunological evaluation of novel*Mycobacterium tuberculosis* culture filtrate proteins. FEMS Immunology and Medical Microbiology 23, 159-164.

Weldingh, K., Rosenkrands, I., Jacobsen, S., Rasmussen, P.B., Elhay, M.J. & Andersen, P. (1998). Two-dimensional electrophoresis for analysis of *Mycobacterium tuberculosis* culture filtrate and purification and characterization of six novel proteins. Infection and Immunity 66, 3492-3500.

Xu, J., Laine, O., Masciocchi, M., Manoranjan, J., Smith, J., Du, S.J., Edwards, N., Zhu, X., Fenselau, C. & Gao, L.Y. (2007). A unique Mycobacterium ESX-1 protein co-secretes with CFP-10/ESAT-6 and is necessary for inhibiting phagosome maturation. Molecular Microbiology 66, 787-800.

Zhang, Y., Lathigra, R., Garbe, T., Catty, D. & Young, D. (1991). Genetic analysis of superoxide dismutase, the 23 kilodalton antigen of *Mycobacterium tuberculosis*. Molecular Microbiology 5, 381-391.



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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