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Dissection of Relationship between Small Heat Shock Proteins and Mycobacterial Diseases

B Jee*, VM Katoch**, SK Awasthi***

Abstract

Mycobacteria belong to a genus which has membership ranging from saprophytes to deadly pathogens that cause several infectious diseases affecting a large population of the world. Among them, tuberculosis and leprosy are the major granulomatous mycobacterial diseases. While there are successes and failures in the fight against these infections, mechanisms of pathogenesis continue to be a challenge to clinicians and biologists alike. Though it is known that both host and bacterial factors are important in the pathogenicity versus protection, all the triggers and responses are not known. Among various bacterial factors, small heat shock proteins (sSHPs) could be important targets for drug development, immunomodulation and serodiagnosis. sSHPs are the molecular chaperones that are believed to act as mantle for the mycobacteria against host's immune attack and facilitate the survival of pathogen in host body. Best studied small heat shock proteins in *M. tuberculosis* are sSHP16.3 and Acr2 while in *M. leprae*, it is 18 kD protein antigen. In this review, works on various aspects of small heat shock proteins which fall in 10 to 19 kD range have been summarized and some thoughts about future road-map have been put into.

Key words: Small heat shock proteins, sSHP 16.3, Acr2, Mycobacterial diseases

Introduction

In the hierarchy of microbial evolution, mycobacteria represent a distinct group of microbes. This single genus mycobacterium has more than 130 known species and 11 subspecies out of which nearly one third are to be associated with human diseases (Katoch 2004, Katoch et al 2007, Euzeby 2008). Mycobacteria are acid fast, have a lipid rich cell wall and high GC contents. Various attempts have been made to taxonomically classify the mycobacteria on the basis of growth rate, pigmentation, nutritional requirements, pathogenesis and virulence (Runyon 1959, Goodfellow et al 1982). Taxonomic classification shows genus

* B Jee, MSc, Research Scholar, National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Dept of Health Research, Ministry of Health and Family Welfare, Govt of India, Dr M Miyazaki Marg, Tajganj, Agra-282001; Institute of Life Sciences, CSJM University, Kanpur-208024, India

** VM Katoch, MD, Director, National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Dept of Health Research, Ministry of Health and Family Welfare, Govt of India, Dr M Miyazaki Marg, Tajganj, Agra-282001, India

*** SK Awasthi, PhD, Assistant Professor, Institute of Life Sciences, CSJM University, Kanpur-208024, India

Correspondence to : Dr VM Katoch, Email:vishwamohan_katoch@yahoo.co.in

mycobacterium is very close relative of some other genera such as *Nocardia, Rhodococcus,* and *Corynebacterium* (Holt et al 1994). *Mycobacterium tuberculosis*, *Mycobcterium leprae* and *Mycobacterium bovis* are among the slow-growing obligate human pathogens which have been extensively studied in past two decades (Tappeiner and Wolff 1999). Among more than 40 mycobacterial species that have been reporeted to cause infections in humans, imporatnt slow growers include *Mycobacterium intracellulare* on one hand and *Mycobacterium fortuitum* and *Mycobacterium chelonae* (rapid growers) on other hand (Katoch 2004, Katoch et al 2007). *Mycobacterium tuberculosis* (MTB) and *Mycobacterium leprae* predominantly reside in macrophages and Schwann cells are the etiological agents of tuberculosis and leprosy respectively.

The virtual hallmark of tuberculosis, leprosy and other diseases caused by slow growing mycobacteria is the formation of granuloma in the infected host. It is thought that granulomas are formed in the host system in response to stimulus by components of persistent intracellular pathogens and finally resulting from accumulation of macrophage-derived epitheloid histocytes, Langhans' giant cells. These cells are further circumscribed by layers of lymphocytes and extracellular matrix. The process may result in necrosis which is generally associated with formation of caseous granulomas (Tappeiner and Wolff 1999, Peters and Ernst 2003). Understanding the evolution of mycobacterial granuloma is very important in for improving the treatment and also for finding newer tools for prevention of diseases.

An important feature of evolution of tuberculosis as a disease is the prolonged latency during which its causative pathogen is able to survive for long periods sometimes in inflamed and necrotic tissues in pulmonary granulomas (Dannenberg 1993, Wayne 1994). The dormancy and persistence are thought to be linked to reactivation of disease / relapses. However, the mechanisms concerned to the entry, persistence, dormancy, viability and reactivation of *Mycobacterium tuberculosis* and the factors facilitating the pathogenesis of tuberculosis are still not fully understood. Similarly, picture has not been clear in the case of leprosy.

After unravelling the complete genome sequence of *M. tuberculosis* and *M. leprae* by Cole and coworkers in 1998 and 2001, new avenues have been opened in the mycobacteriology which could provide a deep insight into the understanding the mechanisms and factors associated with the development of mycobacterial diseases. How mycobacteria survive inside the granulomas or caseous lesions, what are the responsible factors for persistence and dormancy, what are the virulence factors and in what way mycobacteria nullify the antimicrobial action of host and which biomolecules and genes contribute in the host-pathogen interaction can be better dissected with new knowledge about genomes of these pathogens, humans and also experimental hosts like guinea pigs / mouse.

A large amount of data has been generated about host and bacterial factors involved in the virulence / pathogenesis mechanisms of mycobacterial diseases. It is thought that when *M. tuberculosis* enters into the macrophages, specific protective immune system of host is activated. As a consequence, various effector molecules like IFN-γ and TNF-α are released which synergistically govern the release of reactive oxygen and nitrogen intermediates (ROIs and RNIs) such as H_2O_2 and NO. While the physiological significance of ROIs in the protection of host against MTB is well established, the role of

ROI is not fully clear. In addition to these mediators, Toll Like Receptors (TLRs) are important factors that take part in the innate immunity (Flynn and Chan 2001). An interplay of Th1 (IFN-γ ,TNF-α, IL-2) and Th2 (IL-4, IL-I0) mediated cytokines is believed to have important roles in the development of cell mediated immune response against the *M. tuberculosis's* attack (Orme et al 1993, Rook and Hernandez-Pando 1996). *In vitro* and *in vivo* studies shows that a wide range of chemokines such as MCP-I, MCP-3, MCP-5, MIPI-α, MIP-β MIP-2, IP-10 and RANTES are produced when immune cells encountered *M. tuberculosis* (Orme and Cooper 1999). Not only the immunological components contribute in the localization or spread of these infections within the body, various genes and their products are also a major regulator of pathogenesis. Several bacterial genes have been identified that can affect the cascade of infection largely. Amongst these genes, *eis* (Wei et al 2000), *hspX* (Yuan et al 1996), members of two component system (TCS) (Cole et al 1998), sigma factors (Manganelli et al 1999, 2004) are most important candidates which facilitate the survival of MTB within macrophages. There is a long list of hypothetical virulence factors which may be directing the degree of host-pathogen interaction and virulence of MTB inside the host. Members of ESAT-6 family, Antigen 85 Complex, LAM are the well characterized virulence factors while HbhA, OmpA , IdeR are highly suspected virulence factors of MTB whose precise role has to be determined (Smith 2003). Laminin-binding-protein (LBP) is an important virulence factor of *M. leprae* (Shimoji et al 1999). In this context, small heat shock proteins (sHSPs) whose molecular weight ranges from 10-19 kD are considered promising molecules that may protect the pathogens against the killing attack of host (Narberhaus 2002, Macario and Macario 2005). These may be targeted to develop new strategies for the termination of life cycle of mycobacteria and could be used in anti-TB drug development. This review concisely examines the inherent relationship between small heat shock proteins and mycobacterial diseases.

Small heat shock proteins: Structure and Function

Small heat shock proteins (sHSPs) are among the least understood molecular chaperones which have attracted considerable attention from microbiologists and molecular biologists over the past decade because of their promising features and apparent functions. sHSPs are ubiquitous in nature and widely found in both prokaryotes and eukaryotes as surface antigens forming one of the major groups of heat shock proteins or stress proteins (Lindquist and Craig 1988). Heat shock and other form of stress are key factors which may modulate the expression of sHSPs genes to a great extent (Morimoto et al 1990). Members of sSHPs protein family are characterized by evolutionarily conserved alpha- crystallin domains which consist of a stretch of 90-100 amino acid residues (de Jong et al 1993). It is hypothesized that this alpha-crystallin domain has many β-strands and form the classical seven - β-strand Ig like fold due to which it has shown close proximity with the immunoglobulin superfamily (Mornon et al 1998). The sequence analysis of sHSPs shows that the conserved crystallin domain is flanked by a highly variable N-terminal region and a more conserved short C-terminal extension (Auqusteyn 2004). Molecular mass analysis of sSHPs revealed that sHSP monomers range from 12 to 43 kD (Narberhaus 2002) and they assemble into a large oligomeric complex consisting of 9 to >30 subunits *in vivo* and *in vitro* and it depends on the class of sSHP (vanMontfort et al 2001). The family sHSPs are much less

conserved than the other families of HSPs (such as HSP60, 70 and 90) (de Jong et al 1993). Available evidences suggest that the sHSPs are functionally molecular chaperones facilitating the suppression of aggregation of denaturing proteins and their refolding during the course of stress conditions (Table 1 and Table 2) (Narberhaus 2002) but what will be their physiological and clinical significance is yet to be answered. It has been hypothesized that the members of α-crystallin family are important for the maintenance of eye lens transparency and prevent the cataract in vertebrates (Brady et al 1997) as well as play a significant role in the regulation of programmed cell death (Bruey et al 2000).

Small and Heat Shock Proteins and *Mycobacterium tuberculosis*

Mycobacterium tuberculosis has two small heat shock proteins : Acr1 (α-crystallin related protein 1 or HSP16.3/16 kD antigen/HspX) encoded by gene *hspX* and Acr2 (HrpA) encoded by *acr2* respectively. Various factors have been identified which affect the expression of both small heat shock protein encoding genes (Table 2) (Kennaway et al 2005). In the coming section the importance of both these genes has been discussed separately.

Small Heat Shock Protein 16.3 (sHSP16.3) /Acr 1

Mycobacterium tuberculosis small heat shock proteins 16.3 (MTB sHSP16.3) was initially identified as a 14 kD immuno-dominant antigen (Verbon et al 1992). Later it has been characterized to be a molecular chaperone that prevents the aggregation of denaturing proteins and misfolding of nascent peptides under different stress conditions (Chang et al 1996). sHSP16.3 is a stable protein (Hu and Coates 1999) belongs to the α - crystallin family or α - heat shock protein (α-HSP) superfamily (Valdez et al 2002). It is synthesized at a low level in logarithmic or exponential-phase cultures, but its synthesis increases markedly during the transition from log phase to stationary phase. This protein becomes one of the most abundant proteins in stationary- phase MTB (Yuan et al 1996).

The cellular localization of MTB sHSP16.3 is unknown till date, although it has been identified as a major membrane protein (MMP) having lipophobic properties (Lee et al 1992), which seems to be present outside of the cell wall of mycoabcterium (Schoningh et al 1990, Verbon et al 1990). It is not a secreted protein (Abou-Zeid et al 1988). After performing the electron microscopic study, Cunningham and Spreadbury (1998) suggest that sHSP 16.3 may be playing a major role of in the cell wall thickening and could be used as a drug target.

The structural organization of sSHP16.3 has been extensively studied. Many-a-study reveal that sSHP16.3 uses trimer as a building block of its synthesis and exists as nonameric complex consisting of trimers of trimers whose calculated molecular mass is 16,277 D (Chang et al 1996, Abulimiti et al 2003). This is consistent with the earlier report made by Kolk et al (1989) which described molecular mass of 14 kD protein is to be 16,000 D. On the other hand, Kennaway et al (2005) reported that Acr1 is a dodecameric assembly formed from a tetrahedral arrangement of monomers and dimer is the building block of its constitution. MTB sHSP16.3 has significant homology with proteins of alpha-crystallin superfamily and is composed of 144 amino acid residues (Verbon et al 1992). Like other members of sHSPs/alpha-crystallin superfamily proteins, MTB sHSP16.3 has characteristic α-crystallin conserved domain of about 85 residues long which is flanked by a nonconserved N-terminal region of about

Table 1 : Heat shock proteins (HSPs) of different mycobacterial species and their reported function(s)

Heat shock protein			Organism	Accession number	Size (AA)*	Function(s)
Protein name	Gene name	Synonym(s)				
HSP10	*groES*	CPN10/groES protein/10 kDa antigen/10 kDa chaperonin/ BCG-A heat shock protein/ Protein Cpn10	*Mycobacterium tuberculosis*	Rv3418c	100	Co-chaperone in HSP60/10 protein folding machinery, associated with autoimmunity/inflammation[#]
			Mycobacterium bovis	Mb3452c	100	
			Mycobacterium avium	MAV_4366	100	
			Mycobacterium leprae	ML0380	100	
HSP60	*groEL1*	CPN60.1/groEL protein 1/60 kDa chaperonin 1/Protein Cpn 60-1	*Mycobacterium tuberculosis*	Rv3417c	539	Molecular chaperone, associated with autoimmunity/inflammation[#]
			Mycobacterium bovis	Mb3451c	539	
			Mycobacterium avium	MAV_4365	538	
			Mycobacterium leprae	ML0381	537	
HSP65	*groEL2*	CPN60.2/groEL protein 2/60 kDa chaperonin 2/Cell wall protein A/65 kDa antigen/ Antigen A/Heat shock protein 65/Protein Cpn 60-2	*Mycobacterium tuberculosis*	Rv0440	540	Molecular chaperone, associated with autoimmunity/inflammation[#]
			Mycobacterium bovis	Mb0448	540	
			Mycobacterium avium	MAV_4707	541	
			Mycobacterium leprae	ML0317	541	
HSP70	*dnaK*	Heat shock 70 kDa protein/ Chaperonin protein dnaK/ Heat shock protein 70/DnaK	*Mycobacterium tuberculosis*	Rv0350	625	Molecular chaperone with ATPase activity, associated with autoimmunity/inflammation[#]
			Mycobacterium bovis	Mb0358	625	
			Mycobacterium avium	DNAK_MYCA1	623	
			Mycobacterium leprae	ML2496	620	
HSP90	*htpG*	High temperature protein G/ Heat shock protein htpG/ HSP90 family protein/ Chaperone protein htpG/HtpG	*Mycobacterium tuberculosis*	Rv2299c	647	Molecular chaperone with ATPase activity
			Mycobacterium bovis	Mb2321c	647	
			Mycobacterium avium	MAV_2118	644	
			Mycobacterium leprae	ML1623	656	
ClpB	*clpB*	Heat shock protein F84.1/ Endopeptidase ATP-binding protein (chain B)	*Mycobacterium tuberculosis*	Rv0384c	848	Probably recovers the cell from heat-induced damage and essential for *in vivo* survival and pathogenicity
			Mycobacterium bovis	Mb0391c	848	
			Mycobacterium avium	MAV_4793	848	
			Mycobacterium leprae	ML2490	848	

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ClpC1	*clpC1*	Probable ATP-dependent protease ATP-binding subunit ClpC1	*Mycobacterium tuberculosis*	Rv3596c	848	Involved in degradation of denatured protein
			Mycobacterium bovis	Mb3627c	848	
			Mycobacterium avium	MAV_0556	822	
			Mycobacterium leprae	ML0235	848	
ClpC2	*clpC2*	Possible ATP-dependent protease ATP-binding subunit ClpC2	*Mycobacterium tuberculosis*	Rv2667	252	Unknown
			Mycobacterium bovis	Mb2686	252	
			Mycobacterium avium	MAV_3559	250	
DnaJ1	*dnaJ1*	Chaperone protein dnaJ1	*Mycobacterium tuberculosis*	Rv0352	395	Prevents aggregation of stress-denatured proteins under heat shock condition
			Mycobacterium bovis	Mb0360	395	
			Mycobacterium avium	MAV_4806	392	
			Mycobacterium leprae	ML2494	388	
			Mycobacterium chelonae	A7BJ16_MYCCH	394	
DnaJ2	*dnaJ2*	Chaperone protein dnaJ2	*Mycobacterium tuberculosis*	Rv2373c	382	Prevents aggregation of stress-denatured proteins under heat shock condition
			Mycobacterium bovis	Mb2394c	382	
			Mycobacterium avium	MAV_2023	381	
			Mycobacterium leprae	ML0625	378	
HspR	*hspR*	Transcriptional regulator HspR/HSPR	*Mycobacterium tuberculosis*	Rv0353	126	Transcriptional regulator (Repressor) of heat shock proteins
			Mycobacterium bovis	Mb0361	126	
			Mycobacterium avium	MAV_4805	131	
			Mycobacterium leprae	ML2493	132	
HtpX	*htpX*	Probable protease *htpX* homolog / EC 3.4.24.-	*Mycobacterium tuberculosis*	Rv0563	286	Involved in hydrolysis of peptides/proteins
			Mycobacterium bovis	Mb0578	286	
			Mycobacterium avium	MAV_4580	287	
			Mycobacterium leprae	ML2278	287	
GrpE	*grpE*	HSP-70 cofactor/Protein grpE	*Mycobacterium tuberculosis*	Rv0351	235	Co-chaperon in HSP70 associated protein folding machinery
			Mycobacterium bovis	Mb0359	235	
			Mycobacterium avium	MAV_4807	227	
			Mycobacterium leprae	ML2495	229	

Resource : http://genolist.pasteur.fr/TubercuList, http://genolist.pasteur.fr/Leproma, http://genolist.pasteur.fr/BoviList, http://www.expasy.org/uniprot, http://www.genome.jp/kegg, # van Eden 2003

*__AA__ : Amino Acid

Table 2 : Characteristic features of major mycobacterial small heat shock proteins (sHSPs)

Small heat shock protein	Synonym(s)	Encoding gene	Organism	Mol. weight (Dalton)	Localization	Size (Amino acid)	Induction	Function(s)	Remarks
Acr1	sHSP16.3/ HSP16.3/14 kD antigen/16 kD antigen/HspX/ α-crystallin related protein 1	*hspX/acr/ acr1/* Rv2031c	*M. tuberculosis*	16277	Cell wall/ membrane	144	Hypoxia (Oxygen deprivation)/ RNIs	i. Molecular chaperone ii. Cell wall thickening	Associated with dormancy
Acr2	HrpA	*hsp/acr2/* Rv0251c	*M. tuberculosis*	17786	Membrane/ Ribosome fractions	159	Heat/uptake by macrophage/ H_2O_2/SDS/ High dose NO/ Palmitic acid	Molecular chaperone	Associated with pathogenesis
18 kD antigen	HSP16.7	*hsp18/* ML1795	*M. leprae*	16707	Cytosol	148	ND*	Not known	Major immune reactive protein

* **ND** = No data yet available

41 residues and followed by a more conserved C- terminal extension of about 16 residues long stretch (Leroux et al 1997, Narberhaus 2002).

The chaperone activity is a characteristic feature and an important function of sHSPs which has been extensively studied during the last two decades. Initially alpha-crystallin protein was investigated for its chaperone function. Later various attributes of MTB sHSP16.3 were studied. The first work on chaperone activity of MTB sHSP16.3 was published by Chang and coworkers in 1996. They tested this heat shock / stress induced protein for its chaperone activity using pig heart citrate synthase (CS) as a substrate. The study clearly indicates that the 16 kD antigen can function as molecular chaperone *in vitro* by inhibiting the heat induced aggregation of citrate synthase at 39.5°C effectively. Several studies showed that chaperone activity of MTB sHSP16.3 is exclusively temperature dependent. A phase change in sSHP16.3 occurs at approximately 60°C. This change directs the removal of a structural energy barrier which eventually enhances the functioning of chaperone machinery (Mao et al 2001). The chaperone activity of MTB sHSP16.3 is also affected in a range of physiological temperatures (25 to 37.5°C) but during such condition, its native oligomeric complexity is not affected. Moreover, with the elevation of temperature, sHSP16.3 nonamer exposes its higher hydrophobic surfaces (Fu and Chang 2004) and after dissociation into smaller momomers, accelerates the chaperone like activity by binding aggregation - prone substrates (Fu et al 2003). These are evidences which show that the chaperone activity of MTB sHSP16.3 is independent of the effects of ATP (Chang et al 1996, Yang et al 1999), however, Valdez and coworkers (2002) reported that ATP plays a pivotal role in the chaperone activity of MTB sHSP16.3 by protecting it from proteolytic attack of chymotrypsin and ATP enhances the chaperone effect by two fold. Despite this information, the biological importance of chaperone modulation by ATP influx and temperature is still unknown.

Kingston et al (1987) were the first to study the immunological activity of recombinant 14 kD antigen of MTB. They showed that 14 kD antigen is capable of generating strong cell mediated immune (CMI) response and induces delayed type hypersentivity (DTH) reaction in mice and guinea pigs model. Several works have been carried out in the past to test the diagnostic accuracy of 16 kD antigen. Results show that the generation of humoral immune response by this antigen may be implicated in the detection of latent tuberculosis (Beck et al 2005). Some workers have studied the antigenicity and cross reactivity of this protein antigen (Jurcevic et al 1996, Wilkinson et al 1998) which showed that 16kD has at least four distinct B-cell epitopes localized within the three regions (Verbon et al 1992). Timm et al (2006) showed that multidrug-resistant *acr1*-deficient clinical isolate of *Mycobacterium tuberculosis* is unimpaired for replication in macrophages.

Undergoing the dormancy and subsequent survival of *M. tuberculosis* in tuberculosis infection is a major impediment to treat this deadly disease effectively. It is well known fact that during the dormancy, drugs fail to function completely (Dickinson and Mitchison 1981). Because of this reason, the dormant *M. tuberculosis* and its survival and persistence despite multi drug therapy (MDT), have drawn proper attention of researchers. Several works have been done in past few years to study the survival mechanisms of MTB during dormancy. Findings clearly showed that sHSP16.3 is a potentially important component which

facilitates the survival of *M. tuberculosis* during prolonged periods of infection (Yuan et al 1996, 1998; Hu and Coates 1999).

M. tuberculosis sHSP16.3 is encoded by gene *hspX* which is also known as Rv2031c. Its standard name is *acr* (Cole et al 1998). The effect of various factors on expression of *hspX* gene has been studied. One of these factors is hypoxia or oxygen deprivation and it has been shown that sHSP16.3 is a hypoxia induced proteins and accumulates during infection of macrophages (Yuan et al 1996, 1998; Cunningham and Spreadbury 1998, Sherman et al 2001). Desjardin and co-workers (2001) suggested that *hspX* is induced in aerobic conditions. In addition, reactive nitrogen intermediates (RNIs) has also been reported to induce expression of this gene to a greater extent (Garbe et al 1999). All this data indicate that *hspX* is hypoxia and RNI-induced gene that may be regulated via overlapping signalling pathways. Despite this important knowledge, actual mechanisms of activation are still unknown (Ohno et al 2003). A series of experiments showed that *dosR/devR* is a two component regulatory system that controls the expression of *hspX* gene during the hypoxic conditions (Das Gupta et al 2000, Sherman et al 2001). It has also been proposed that *hspX* gene has only one operon including a putative two component transcription regulator *dosR/devR* (Sherman et al 2001). Further studies showed that the gene *hspX* of *M. tuberculosis* is present in a locus which comprises of two USPs namely the carbohydrate kinase and a DevS like sensor kinase, Rv2027c (O'Toole et al 2003). Before the discovery of two component regulatory system, it was thought that stress-responsive sigma factor *sigF* is the key regulator of the expression of MTB sHSP16.3 (Manabe et al 1999) while Sherman and coworkers (2001) reported that *sigF* is not the sole regulator of gene *hspX*. One interesting study shows that deletion of gene *hspX* of *M. tuberculosis* causes increased bacterial growth *in vivo* (Hu et al 2006). In contrast, deletion of *dosR* (the gene that controls *hspX*) has been reported to cause hypervirulence in mouse models and in activated macrophages (Parish et al 2003).

Small Heat Shock Protein Acr2

Acr2 is another novel member of the α-crystallin family of molecular chaperones and small heat shock protein of *M. tuberculosis* that is encoded by gene *acr2* (Rv0251c / *hrpA* / *hsp20*) (Stewart et al 2002). *M. bovis* also possesses an *acr2* gene identical with that of *M. tuberculosis* H37Rv (Garnier et al 2003).

The 18 KD small heat shock protein Acr2 which is also referred to as HrpA (Heat stress induced ribosome binding protein A) has been detected in the ribosomal fractions of *M. bovis BCG* when subjected to heat treatment (Ohara et al 1997). Several lines of evidences indicate that the regulation of *acr2* is multifactorial and complex. Various factors have been studied which significantly induced the expression of *acr2*, amongst which sodium dodecyl sulfate (SDS), starvation conditions, palmitic acid, uptake by naive and activated macrophages and oxidative stress produced by exposure to diamide or hydrogen peroxide and heat shock at 45°C are prominent (Manganelli et al 2001, Schnappinger et al 2003). On the basis of induction studied using different factors, *acr2* has been included in a group of seven *M. tuberculosis* genes that are significantly up-regulated in response to multiple stresses. The expression of this key protein Acr2 was significantly down-regulated by the heat shock repressor protein HspR (Stewart et al 2002) and by a two-component system (TCS) called *phoPR* during the logarithmic growth in liquid medium (Walters et al 2006) whereas

SigE and SigH are alternative sigma factors that down-regulates the expression of *acr2* (Manganelli et al 2002, Raman et al 2001). Recent studies by Pang and Howard (2007) have shown that the expression of *acr2* may positively or negatively be regulated by a two-component system *mprAB* in *M. tuberculosis*. Homology studies show that Acr2 has 30% amino-acid sequence similarity to the Acr1/sHSP16.3 encoded by *hspX*/*acr*/Rv2031c of *M. tuberculosis*. The quantum of similarity can increase to 41 % depending upon comparison of residues present in the core of α-crystallin domain (Stewart et al 2002).

The biological role of Acr2 is not completely known. However, several efforts have been made. In the sequence of experiments, Ohara and co-workers (1997) showed that Acr2 may promote the stabilization of 30S subunit of the ribosome at elevated temperature and thereby facilitates initiation of translation. It was observed that α-crystallin 2 (*acr2*) is invariably associated with pathogenesis of *M. tuberculosis* infection and is expressed at a high level in the mouse model during both acute and chronic infection (Stewart et al 2005). Further, deletion of *acr2* gene was reported to result in decrease in the resistance of MTB to oxidative stress but there is no impairment in growth of bacilli has been observed in mouse bone marrow derived macrophages. These findings demonstrate that both α-crystallins (Acrl and Acr2) contribute to pathogenesis and persistence of tubercle bacilli (Stewart et al 2005). Since expression of Acr2 is up regulated just after entry of *M. tuberculosis* into host cells in response to exposure of host reactive oxygen intermediates, Acr2 is postulated as an early immune target that can contribute in the early recognition of infection by host (Wilkinson et al 2005).

Small Heat Shock Proteins and other Mycobacteria

Small heat shock proteins which have been reported in other mycobacteria viz. *M. leprae*, *M. avium* and *M. intracellulare* are *acr* homologous whose molecular weight ranging from 10 to 19 kD and show good immunogenecity. In *M. leprae*, presence of several antigens that act as small heat shock proteins have been shown. 10 kD sSHP is the one of major T-cell antigen of *M. lepare* which stimulates peripheral blood T-cells to produce high levels of antibodies against itself (Mehra et al 1992, Rojas et al 1997). 15 kD is another protein antigen identified by Vega-Lopez and coworkers (1988) in the sera of patients suffering from leprosy. In the continuation of the work on sHSPs in other mycobacteria, Nerland and his group (1988) have identified an 18 kD protein antigen from *M. leprae* which also belongs to small heat shock proteins family and bears 30% sequence identity to the soyabean Hsp18 and 27% to 16 kD antigen of *M. tuberculosis* (Lee et al 1992). Experimental evidences shows that 18 kD protein antigen is present in *M. leprae* as well as in *M. habana* (now considered to be *M. simiae* serovar 1) (Lamb et al 1990). *M. kansasii*, *M. terrae*, *M. avium*, *M. scrofulaceum*, *M. gordonae*, *M. chelonei* and *M. intracellulare* seem to possess proteins with homologous sequences (Moudgil et al 1992). 18 kD recombinant protein from the *M. leprae* have been characterized by Hussain et al (1992). Booth and coworkers (1993) showed the presence of homologous of 18 kD antigen of *M. leprae* and 19 kD antigen of *M. tuberculosis* in other mycobacteria like *M. avium* and *M. intracellulare*. Like other mycobacterial antigens, 18 kD protein appears to be a potent stimulator of CD4+ T cell responses, due to having epitopes that are antigenic to T cells (Mustafa et al 1986, 2000) and shows MHC Class II-restricted cytotoxicity (Adams et al 1995).

Future Prospective

It is apparent that stress induced proteins or small heat shock proteins (sHSPs) of pathogenic mycobacteria act as molecular chaperones and are important for the proper assembly and refolding of nascent polypeptide chain and also for translocation of proteins across subcellular membranes to their appropriate cellular compartments. While significant amount of data about their chaperone action, induction under hypoxic conditions and RNIs induced conditions has been generated, very little attention has been paid to explore their therapeutic relevance. Keeping the current situation in mind there is an need to target sSHP16.3 for the discovery of new cost effective anti-mycobacterial drugs / immunomodulatory agents. These could also be developed as an efficient diagnostic targets. However, this demands an interdisciplinary approach to study an inherent phenomenon occurring during the interaction between the sHSPs and host machinery. Advances in the techniques related to functional genomics and proteomics as well as immunology will be helpful in taking the process forward which merits serious attention.

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