Indian Journal of Experimental Biology
Vol. 47, June 2009, pp. 401-406

Review Article

Interference of *Mycobacterium tuberculosis* with macrophage responses

Nicole Scherr, Rajesh Jayachandran, Philipp Mueller & Jean Pieters*
Biozentrum, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland

Tuberculosis, caused by *Mycobacterium tuberculosis,* has become an important health and economic burden, with more than four thousand people succumbing to the disease every day. Thus, there is an urgent need to understand the molecular basis of this pathogen's success in causing disease in humans, in order to develop new drugs superior to conventional drugs available at present. One reason why *M. tuberculosis* is such a dangerous microbe lies within its ability to survive within infected hosts, thereby efficiently circumventing host immune responses. Over the past few years, a number of mechanisms have been unravelled that are utilized by *M. tuberculosis* to survive within hosts and to avoid immune defence mechanisms. Several of these mechanisms have been described in this communication that may be useful for the development of novel compounds to treat tuberculosis.

Keywords: Immune defence, Macrophage response, *Mycobacterium tuberculosis*

Introduction

Tuberculosis belongs to the deadliest infectious diseases worldwide. Approximately one third of the global population is infected with *M. tuberculosis,* 10% of these individuals develop active tuberculosis and die. New drugs and treatments are of urgent need, especially with respect to the emergence of multi-drug resistant and extensive drug resistant strains, which are virtually untreatable and cause a high lethality in infected individuals[1]. Whereas normally, the host immune system is capable to destroy invading bacilli, *M. tuberculosis* has developed a plethora of strategies to counteract the bactericidal activities of the host[2].

In general, upon internalization by macrophages, bacteria are enclosed in a specialized vacuole, the phagosome[3]. During the maturation of the phagosome, fusion and fission events take place, which gradually cause the phagosome to mature into or fuse with lysosomes, in which efficient degradation of cargo is ensured by the activity of various destructive mechanisms[4, 5].

However, pathogenic mycobacteria possess the capacity to modulate their own trafficking route by inhibiting phagosome-lysosome fusion and to create an intracellular niche allowing them to survive for prolonged periods inside the host[6]. Mycobacterial phagosomes are characterized by the absence of a specific set of late endosomal or lysosomal markers such as Rab7, v-ATPase, LAMP1 and the two PI(3)P binding proteins EEA1 (early endosome antigen 1) and Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate)[7,8]. *M. tuberculosis* utilizes several protein as well as lipid molecules to arrest phagosome maturation at an early stage[2,9,10]. Host proteins and bacterial factors which enable pathogenic mycobacteria to modulate trafficking of phagosomes and ensure their survival are listed in Fig. 1 and discussed below.

Mycobacterial factors

Mycobacteria prevent the accumulation of phosphatidyl-inositol-3-phosphate [(PI(3)P)], an essential component of phago-lysosome biogenesis, at the phagosomal membrane. As a result, PI(3)P-dependent recruitment of the late endosomal markers Hrs and EEA1 via PI(3)P is abolished and consequently the phagosomes fail to fuse with lysosomes[11,12]. One of the factors responsible for

Macrophage	*M. tuberculosis*
Calcineurin	Esx-1
Coronin 1	LAM
LRG 47	PknG
	PtpA
	SapM

Fig. 1—Factors modulating phagosome trafficking.

*Correspondent author
Telephone: 00 41 61 267 14 94; Fax: 00 41 61 267 21 48
E-mail: jean.pieters@unibas.ch

reduced levels of PI(3)P on the phagosomal vacuole is lipoarabinomann (LAM), a lipoglycan of the mycobacterial cell wall. LAM inhibits the activity of the PI3 kinase hVP34 that generates PI(3)P on the phagosomal membrane, eventually resulting in the arrest of phagosome maturation[13]. Another molecule inducing the reduction of PI(3)P is an eukaryotic-like acid phosphatase termed SapM, produced by mycobacteria and secreted within the host cell[14]. SapM hydrolyzes PI(3)P accumulated on phagosomal membranes, thereby eliminating the docking site for proteins like Hrs or EEA1 involved in phagosomal maturation[15].

In addition to the lipid phosphatase SapM, a tyrosine phosphatase, termed PtpA, has been identified[16,17] which, upon secretion, dephosphorylates and inactivates the host protein vacuolar protein sorting (VPS) 33B, a regulator of membrane fusion. Inactive VPS33B is no longer capable of generating GTP-activated Rab7, resulting in a phagosome maturation block[17].

For the release of molecules that interfere with host responses, *M. tuberculosis* possesses a specialized protein secretion system, the so-called early secretory antigenic target system 1 (ESX-1). Esx-1, encoded by the RD1 (region of difference 1) genomic region represents a major virulence determinant[18-21] and was also suggested to be linked to modulating phagosome maturation. Infection of macrophages with *M. tuberculosis* displaying defects in Esx-1 dependent secretion results in increased lysosomal delivery and killing of the bacteria[22]. On the other hand, *M. bovis* BCG, which lacks the RD1 region, can still block phagosome maturation, and in addition, the known secreted products ESAT-6 (early secretory antigenic target 6), CFP-10 (culture filtrate protein 10) and EspA[23] have been found to be dispensable with regard to the arrest of phagosome maturation, indicating the existence of other modulators[19,20]. Interestingly, components of the Esx-1 secretion system have been suggested to promote escape of *M. tuberculosis* from the phago-lysosome to the cytosol based on the observation that RD1 mutants were not observed to be transferred to the cytosol[24].

One additional virulence factor promoting survival within host macrophages is protein kinase G (PknG), a conserved member of the eukaryotic-like serine/threonine protein kinase family in mycobacteria[25-28]. Upon infection, PknG is released into the cytosol of macrophages where it prevents lysosomal delivery and degradation (Fig. 2). In contrast to pathogenic mycobacteria, non-pathogenic and fast-growing mycobacteria are immediately killed by bactericidal conditions encountered in lysosomes[28]. Recent data revealed that in these species, expression of PknG is suppressed, possibly through inefficient ribosome binding in the *pknG* upstream region or by interactions with other regulatory factors such as regulatory RNA, or proteins[29].

In case of genomic deletion or chemical inhibition of PknG from pathogenic species, mycobacteria are rapidly transferred to lysosomes. However, the exact mode of action of PknG and the identification of potential interacting host factors or substrates remains to be established[27].

The co-crystallization of PknG with a specific inhibitor allowed the definition of three structural distinct domains: an N-terminal rubredoxin motif (RD) with two CXXC sequences coordinating iron, a two-lobed Hanks-type kinase domain (KD) encompassing the catalytic cleft, and a C-terminal domain with TPR repeats (TPRD, Fig. 2) mediating dimerization[29]. The PknG inhibitor, AX20017, a tetrahydrobenzothiophene of small molecular weight, was identified by screening a large compound library and was found to be a potent and specific PknG inhibitor[28]. In the atomic model of PknG, AX20017 occupies the ATP binding pocket while making specific interactions with residues of the PknG-N-terminus and the kinase domain. Despite its relatively simple structure and the high degree of homology between kinase domains in general, AX20017 does not affect eukaryotic host kinases and shows no adverse effects on kinase-related processes in macrophages underlining the high selectivity of the compound[30,31].

Thus, the potential of PknG to circumvent lysosomal delivery and the identification of a potent inhibitory compound make PknG a promising drug target.

Macrophage factors

Relatively few host factors have been characterized that allow pathogenic mycobacteria to escape lysosomal degradation.

(i) LRG-47, a member of the IFN-γ regulated family of p47 GTPases, which has also been shown to play an important role in the control of trypanosomiasis and salmonellosis, is an important component in the endosomal pathway whose function

is needed for the containment of mycobacterial infection[32-34]. LRG-47 was shown to promote autophagy, wherein the intracellular pathogens along with bacterial products and damaged organelles are sequestered into an autophagosome for degradation[35]. In the absence of LRG-47, mycobacterial phagosomes were found to carry lower levels of v-ATPase which additionally hindered the process of phagosomal maturation by preventing acidification of the mycobacterial phagosome.

(ii) Coronin 1, also known as TACO (tryptophan aspartate containing coat protein) is a protein that has been initially identified to be associated with mycobacterial phagosomes isolated from infected macrophages[36,37]. Coronin 1 is a member of a large family of proteins characterized by the presence of WD repeats and is widely distributed in the animal kingdom[38]. In mammals, coronin 1 is predominantly expressed in cells of the immune system including lymphocytes, macrophages and neutrophils. Coronin 1 possesses 5 WD domains, and contains a C-terminal domain that adopts a coiled coil domain containing alpha helices, which are essential for trimerization[39-41]. The protein is located in the subcortical regions of the cell, and upon phagocytosis of inert cargo, coronin 1 remains associated with the cytosolic surface of the phagosome (Fig. 2). During maturation of phagosomes into lysosomes, coronin 1 is released to the subcortical region. When macrophages are being infected with living, but not

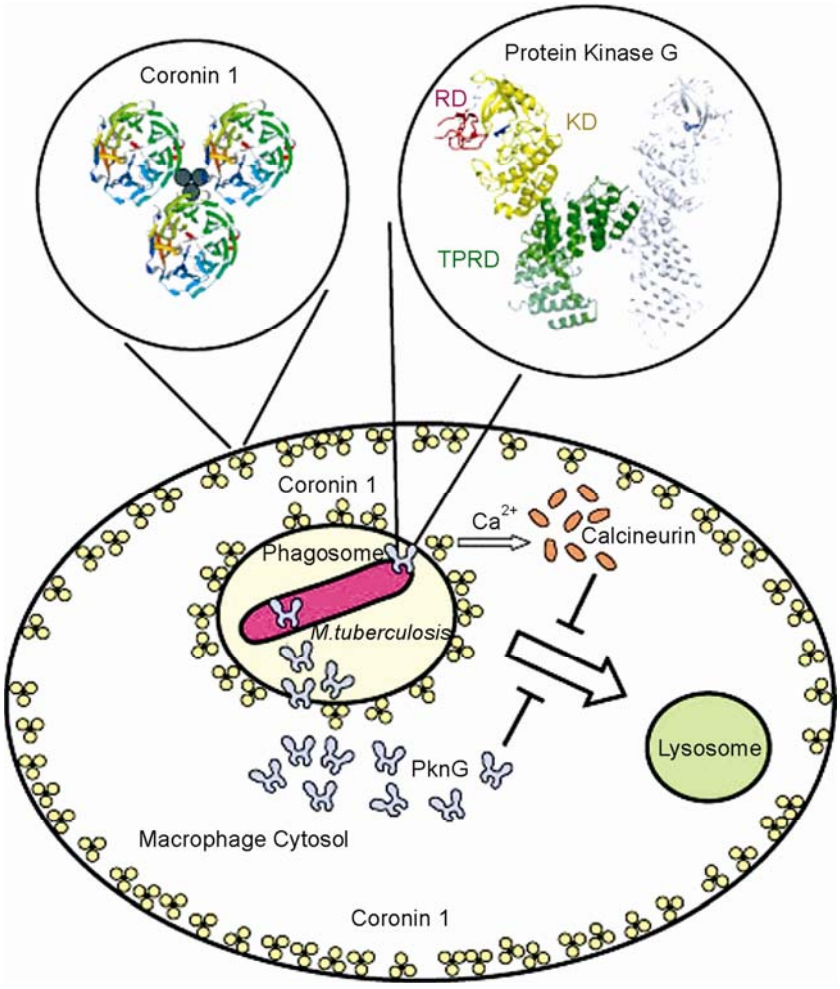

Fig. 2—Role of coronin 1 and protein kinase G (PknG) in phagosome maturation. Following phagocytosis of *M. tuberculosis*, the host protein coronin 1 is recruited to the phagosomal membrane. Coronin 1 triggers the activation of the phosphatase calcineurin which then inhibits phagosome-lysosome fusion. Mycobacterial PknG is secreted into the macrophage cytosol where it prevents lysosomal delivery. Insets: Coronin 1 is a homotrimeric molecule composed of three globular N-terminal β-propellers (containing the WD repeats) assembled via the C-terminal coiled region (reproduced with permission from Appleton *et al*.[38]). PknG forms dimmers each consisting of an N-terminal rubredoxin domain (RD; red), kinase domain (KD; yellow) and C-terminal TPR domain (TPRD; green). The specific inhibitor, AX20017 (blue), binds to the catalytic site and interacts with residues of the N-terminal and kinase domain.

killed pathogenic mycobacteria, the release of coronin 1 from the phagosomes to the subcortical regions is prevented and coronin 1 is retained on mycobacterial phagosomes[36,42].

To investigate the mechanisms of action of coronin 1, coronin 1 deficient animals were generated through targeted gene disruption[43,44]. When macrophages were differentiated from the bone marrow of these animals and the progression of mycobacterial infection was monitored, as expected, the mycobacteria were rapidly delivered to lysosomes resulting in an efficient degradation and killing of the mycobacteria.

While these results confirmed a role for coronin 1 in sustaining *M. tuberculosis* within macrophage phagosomes, they did not reveal the mode of action of coronin 1. Insight into the cellular function of coronin 1 came from an in-depth analysis of the coronin 1 deficient mice. It turned out that in these mice, T cells rapidly disappear from the peripheral vasculature system. Coronin 1 deficient T cells fail to properly transduce T cell receptor signals intracellularly which results in their reduced numbers in the peripheral blood. Specifically, coronin 1 is required to activate Ca^{2+}-dependent signaling reactions, that in normal T cells, via the activation of the Ca^{2+}-dependent phosphatase calcineurin, results in dephosphorylation of the nuclear factor for activated T cells (NFAT), thereby targeting NFAT to the nucleus where it initiates transcription of genes such as interleukin-2 (Ref. 45). These observations than prompted the analysis of a role for coronin 1 in modulating calcium signaling, thereby inducing mycobacterial survival within macrophages. Indeed, in wild type (coronin 1 expressing) macrophages, mycobacterial uptake is associated with an elevation of cytosolic calcium; in the absence of coronin 1, this increase of calcium ions does not occur[2,44]. Thus, it became clear that an increase in cytosolic calcium plays a crucial role in the ability of *M. tuberculosis* to block lysosomal delivery. Indeed, increasing the intracytosolic calcium levels using the calcium ionophore calcimycin prevented lysosomal delivery of mycobacteria even in the absence of coronin 1 (Ref. 45).

One consequence of the increase in cytosolic Ca^{2+} is the activation of the Ca^{2+}-dependent phosphatase, calcineurin[45]. When the cytosol from macrophages infected with *M. bovis* BCG was analyzed for calcineurin activation, it turned out that calcineurin phosphatase activity was significantly enhanced upon infection. In contrast, infection of coronin 1 deficient macrophages with *M. bovis* BCG failed to activate calcineurin, consistent with the absence of Ca^{2+} influx[44].

To corroborate the role of calcineurin in mycobacterial survival, the calcineurin inhibitors cyclosporine A and FK506 were added during infection of wild type macrophages with *M. tuberculosis*. The results showed that all mycobacteria were efficiently killed upon inhibition of calcineurin[44], confirming the important role of calcineurin activation in allowing mycobacterial survival within infected macrophages (Fig. 2). Whether or not calcineurin blockers may be useful for the treatment of mycobacterial infection remains to be established. Also, how and where the interaction occurs between coronin 1 dependent calcium elevation and calcineurin activation still remains to be analyzed. One possibility is that calcineurin is activated locally at the mycobacterial phagosome; alternatively, calcineurin may activate or inhibit a cytosolic factor that modulates phagosome-lysosomal trafficking.

Recently, it has also been shown that lipoamide dehydrogenase secreted from pathogenic strains of mycobacteria could interact by means of cholesterol with coronin 1 thus preventing the release of coronin 1 from the mycobacterial phagosome into the subcortical regions[46], consistent with the earlier demonstrated role for cholesterol in mycobacterial pathogenesis[42].

It is to be expected that the increased knowledge on the molecular basis of the virulence mechanisms utilized by *M. tuberculosis* may provide additional leads for the identification of novel drug targets and for the development of efficient treatments in order to battle tuberculosis.

References

1 Blower S & Supervie V, Predicting the future of XDR tuberculosis, *Lancet Infect Dis*,7(7) (2007) 443.

2 Pieters J, *Mycobacterium tuberculosis* and the macrophage: maintaining a balance, *Cell Host Microbe*, 3(6) (2008) 399.

3 Aderem A & Underhill D M, Mechanisms of phagocytosis in macrophages, *Annu Rev Immunol*,17 (1999) 593.

4 Rohde K, Yates R M, Purdy G E & Russell D G, *Mycobacterium tuberculosis* and the environment within the phagosome, *Immunol Rev*, 219 (2007) 37.

5 Vieira O V, Botelho R J & Grinstein S, Phagosome maturation: aging gracefully, *Biochem J*, 366 (Pt 3) (2002) 689.

6 Armstrong J A & Hart PD, Response of cultured macrophages to *Mycobacterium tuberculosis*, with

observations on fusion of lysosomes with phagosomes, *J Exp Med*, 134 (3 Pt 1) (1971) 713.

7 Vergne I, Fratti R A, Hill P J, Chua J, Belisle J & Deretic V, *Mycobacterium tuberculosis* phagosome maturation arrest: mycobacterial phosphatidylinositol analog phosphatidylinositol mannoside stimulates early endosomal fusion, *Mol Biol Cell*, 15(2) (2004) 751.

8 Chakraborty P, Sturgill-Koszycki S & Russell D G, Isolation and characterization of pathogen-containing phagosomes, *Methods Cell Biol*, 45 (1994) 261.

9 Steinberg B E & Grinstein S, Pathogen destruction versus intracellular survival: the role of lipids as phagosomal fate determinants, *J Clin Invest*, 118(6) (2008) 2002.

10 Philips J A, Mycobacterial manipulation of vacuolar sorting, *Cell Microbiol*, 10(12) (2008) 2408.

11 Fratti R A, Chua J, Vergne I & Deretic V, *Mycobacterium tuberculosis* glycosylated phosphatidylinositol causes phagosome maturation arrest, *Proc Natl Acad Sci U S A*, 100(9) (2003) 5437.

12 Roth M G, Phosphoinositides in constitutive membrane traffic, *Physiol Rev*, 84(3) (2004) 699.

13 Vergne I, Chua J & Deretic V, Tuberculosis toxin blocking phagosome maturation inhibits a novel Ca2+/calmodulin-PI3K hVPS34 cascade, *J Exp Med*, 198(4) (2003) 653.

14 Saleh M T & Belisle J T, Secretion of an acid phosphatase (SapM) by *Mycobacterium tuberculosis* that is similar to eukaryotic acid phosphatases, *J Bacteriol*, 182(23) (2000) 6850.

15 Vergne I, Chua J, Lee H H, Lucas M, Belisle J & Deretic V, Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*, *Proc Natl Acad Sci U S A*, 102(11) (2005) 4033.

16 Grundner C, Gay L M & Alber T, *Mycobacterium tuberculosis* serine/threonine kinases PknB, PknD, PknE, and PknF phosphorylate multiple FHA domains, *Protein Sci*, 14(7) (2005) 1918.

17 Bach H, Papavinasasundaram K G, Wong D, Hmama Z & Av-Gay Y, *Mycobacterium tuberculosis* virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B, *Cell Host Microbe,* 3(5) (2008)316.

18 DiGiuseppe Champion P A & Cox J S, Protein secretion systems in Mycobacteria, *Cell Microbiol*, 9(6) (2007) 1376.

19 MacGurn J A & Cox J S, A genetic screen for *Mycobacterium tuberculosis* mutants defective for phagosome maturation arrest identifies components of the ESX-1 secretion system, *Infect Immun*, 75(6) (2007) 2668.

20 Gao L Y, Guo S, McLaughlin B, Morisaki H, Engel J N & Brown E J, A mycobacterial virulence gene cluster extending RD1 is required for cytolysis, bacterial spreading and ESAT-6 secretion, *Mol Microbiol*, 53(6) (2004) 1677.

21 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, Dissection of ESAT-6 system 1 of *Mycobacterium tuberculosis* and impact on immunogenicity and virulence, *Infect Immun*, 74(1) (2006) 88.

22 Lee W L, Tan T, Alexander D C, Grinstein S & Liu J, The ESAT-6/CFP-10 secretion system of Mycobacterium marinum modulates phagosome maturation, *Cellular Microbiology*, 8(9) (2006) 1417.

23 Fortune S M, Jaeger A, Sarracino D A, Chase M R, Sassetti C M, Sherman D R, Bloom B R & Rubin E J, Mutually dependent secretion of proteins required for mycobacterial virulence, *Proc Natl Acad Sci U S A,* 102(30) (2005) 10676.

24 van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, Brenner M & Peters P J, *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells, *Cell*, 129(7) (2007) 1287.

25 Cole S T, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon S V, Eiglmeier K, Gas S, Barry CE, 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, Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence, *Nature*, 393(6685) (1998) 537.

26 Narayan A, Sachdeva P, Sharma K, Saini A K, Tyagi A K & Singh Y, Serine threonine protein kinases of mycobacterial genus: phylogeny to function, *Physiol Genomics*, 29(1) (2007) 66.

27 Scherr N & Pieters J, The Eukaryotic-like Serine/Threonine Protein Kinase Family in Mycobacteria, In:. *Mycobacterium: Genomics and Molecular Biology,* edited by T Parish and A Brown, (Horizon Press) (in press).

28 Walburger A, Koul A, Ferrari G, Nguyen L, Prescianotto-Baschong C, Huygen K, Klebl B, Thompson C, Bacher G & Pieters J, Protein kinase G from pathogenic mycobacteria promotes survival within macrophages, *Science*, 304(5678) (2004) 1800.

29 Houben E N, Walburger A, Ferrari G, Nguyen L, Thompson C J, Miess C, Vogel G, Mueller B & Pieters J, Differential expression of a virulence factor in pathogenic and non-pathogenic mycobacteria, *Mol Microbiol*, 72(1) (2009) 41.

30 Scherr N, Honnappa S, Kunz G, Mueller P, Jayachandran R, Winkler F, Pieters J & Steinmetz M O, Structural basis for the specific inhibition of protein kinase G, a virulence factor of *Mycobacterium tuberculosis*, *Proc Natl Acad Sci U S A*, 104(29) (2007) 12151.

31 Nguyen L, Walburger A, Houben E, Koul A, Muller S, Morbitzer M, Klebl B, Ferrari G & Pieters J, Role of protein kinase G in growth and glutamine metabolism of Mycobacterium bovis BCG, *J Bacteriol*, 187(16) (2005) 5852.

32 Henry S C, Daniell X, Indaram M, Whitesides J F, Sempowski G D, Howell D, Oliver T & Taylor G A, Impaired macrophage function underscores susceptibility to Salmonella in mice lacking Irgm1 (LRG-47), *J Immunol*, 179(10) (2007) 6963.

33 Feng C G, Collazo-Custodio C M, Eckhaus M, Hieny S, Belkaid Y, Elkins K, Jankovic D, Taylor G A & Sher A, Mice deficient in LRG-47 display increased susceptibility to mycobacterial infection associated with the induction of lymphopenia, *J Immunol*, 172(2) (2004) 1163.

34 MacMicking J D, Taylor G A & McKinney J D, Immune control of tuberculosis by IFN-gamma-inducible LRG-47, *Science*, 302(5645) (2003) 654.

35 Singh S B, Davis A S, Taylor G A & Deretic V, Human IRGM induces autophagy to eliminate intracellular mycobacteria, *Science*, 313(5792) (2006) 1438.
36 Ferrari G, Langen H, Naito M & Pieters J, A coat protein on phagosomes involved in the intracellular survival of mycobacteria, *Cell*, 97(4) (1999) 435.
37 Hasan Z, Schlax C, Kuhn L, Lefkovits I, Young D, Thole J & Pieters J, Isolation and characterization of the mycobacterial phagosome segregation from the endosomal/lysosomal pathway, *Mol Microbiol*, 24(3) (1997) 545.
38 Rybakin V & Clemen C S, Coronin proteins as multifunctional regulators of the cytoskeleton and membrane trafficking, *Bioessays*, 27(6) (2005) 625.
39 Gatfield J, Albrecht I, Zanolari B, Steinmetz M O & Pieters J, Association of the leukocyte plasma membrane with the actin cytoskeleton through coiled coil-mediated trimeric coronin 1 molecules, *Mol Biol Cell*, 16(6) (2005) 2786.
40 Kammerer R A, Kostrewa D, Progias P, Honnappa S, Avila D, Lustig A, Winkler F K, Pieters J & Steinmetz M O, A conserved trimerization motif controls the topology of short coiled coils, *Proc Natl Acad Sci U S A*, 102(39) (2005) 13891.
41 Appleton B A, Wu P & Wiesmann C, The crystal structure of murine coronin-1: a regulator of actin cytoskeletal dynamics in lymphocytes, *Structure*, 14(1) (2006) 87.
42 Gatfield J & Pieters J, Essential role for cholesterol in entry of mycobacteria into macrophages, *Science*, 288(5471) (2000) 1647.
43 Mueller P, Massner J, Jayachandran R, Combaluzier B, Albrecht I, Gatfield J, Blum C, Ceredig R, Rodewald H R, Rolink A G & Pieters J, Regulation of T cell survival through coronin-1-mediated generation of inositol-1,4,5-trisphosphate and calcium mobilization after T cell receptor triggering, *Nat Immunol*, 9(4) (2008) 424.
44 Jayachandran R, Sundaramurthy V, Combaluzier B, Mueller P, Korf H, Huygen K, Miyazaki T, Albrecht I, Massner J & Pieters J, Survival of mycobacteria in macrophages is mediated by coronin 1-dependent activation of calcineurin, *Cell*, 130(1) (2007) 37.
45 Winslow M M, Neilson J R & Crabtree G R, Calcium signalling in lymphocytes, *Curr Opin Immunol*, 15(3) (2003) 299.
46 Deghmane A E, Soualhine H, Bach H, Sendide K, Itoh S, Tam A, Noubir S, Talal A, Lo R, Toyoshima S, Av-Gay Y & Hmama Z, Lipoamide dehydrogenase mediates retention of coronin-1 on BCG vacuoles, leading to arrest in phagosome maturation, *J Cell Sci*, 120(Pt 16) (2007) 2796.