Mycobacterium smegmatis lacking the MspA porin grow defectively due to the lack of glucose uptake but display increased resistance to several antibiotics and also to nitric oxide. Nitric oxide burst is a well described bactericidal mechanism in mouse macrophages and the inducible nitric oxide synthase is the enzyme responsible for NO release. In this study, we describe a novel putative outer membrane protein conserved between *M. tuberculosis* and *Mycobacterium bovis* BCG. We show that the absence of this protein limits bacterial growth *in vitro* but results in increased BCG survival within macrophages. We also demonstrate that although interferon-gamma stimulation of macrophages induces ten times increased killing of BCG, bacteria lacking this protein remain unsusceptible to this stimulation. Furthermore, quantification of iNOS and IL-1beta expression through qRT-PCR revealed that those genes were less upregulated during infection with the mutant bacteria compared to the WT strain suggesting that the increased survival of the mutants is due to lower macrophage activation and release of nitric oxide. We conclude that MtpA from Mtb complex is important to release virulence factors required for macrophage activation.

Acknowlegements

Financial support of FCT project PIC/82859/2007 is gratefully acknowledged.

doi:10.1016/j.drudis.2010.09.437

A92

In vivo phage display to identify peptides that target the brain

Mathew W. Smith*, Mark Gumbleton
Welsh School of Pharmacy, Cardiff CF10 3XF, UK

*Corresponding author.
E-mail: mathewsmith1@me.com (M.W. Smith).

The delivery of novel macromolecular therapeutics into brain parenchyma to treat central nervous system disorders (CNS) is hindered by the blood–brain barrier (BBB). The BBB is comprised of microvascular endothelial cells that line the capillaries traversing the brain. The existence of highly restrictive tight junctions and the relatively low abundance of morphologically evident endocytic vesicles restricts both paracellular and transcellular access to the brain of therapeutic proteins, peptides and nano-medicines [1]. As part of an ongoing programme to identify novel ligands that mediate endocytotic and transcytotic events within the BBB we report here the use of a Phage Display library to identify small cyclic peptides (-7mer) that traverse the *in vivo* rat BBB. A Phage Display Library (Ph.D.-C7C™ New England Biolabs) representing 1.2×10^9 unique genotypes encoding random-7mer disulphide constrained peptides genomically fused to the pIII coat protein of the filamentous phage M13 was utilised in all studies. A synchronous selection strategy [2] was employed to select for peptides homing to a range of organs before undertaking a final selection for peptides that home to brain grey matter. In this final selection the library was injected i.v. into a rat and circulated for 15 minutes before perfusion with saline to remove freely circulating phage and then glycine buffer (pH 2.2) to strip the vasculature of binding phage. The brain was removed and the white matter and capillaries depleted before the grey matter (brain parenchyma) was homogenised and phages recovered. The recovered phages were gene sequenced to determine the corresponding peptide library sequence displayed. From the sequenced population a conserved motif AC-SXTSSTX-CGGGS was identified at a frequency of 25%; secondary phage studies and bioinformatic analysis of a large population of sequenced clones (>500) corroborated this sequence. *In vivo* biodistribution studies of a clone displaying the conserved motif (AC-SYTSSTM-CGGGS) revealed a selective homing to brain grey matter as demonstrated by a 4-fold increase in AUC0-∞ and 3.5-fold increase in Cmax in brain grey matter compared to insertless phage (no displayed phage). Studies are addressing the molecular pathways of entry of this peptide phage into the CNS.

Reference

1. Smith MW, Gumbleton M. Endocytosis at the blood–brain barrier: from basic understanding to drug delivery strategies. *J Drug Target* 2006;**14**:191–214.
2. Kolonin MG, et al. Synchronous selection of homing peptides for multiple tissues by in vivo phage display. *FASEB J* 2006;**20**:979–81.

doi:10.1016/j.drudis.2010.09.438

A93

Phage display identification of a lung transduction peptide that affords enhanced macromolecule transport across the intact lung epithelium

Christopher J. Morris[1,*], Peter Griffiths[2], Neil McKeown[2], Mark Gumbleton[1]
[1] *Welsh School of Pharmacy, Cardiff University, Cardiff, UK*
[2] *School of Chemistry, Cardiff University, Cardiff, UK*

*Corresponding author.
E-mail: morriscj@cf.ac.uk (C.J. Morris).

Evolutionary technologies based upon the screening of combinatorial libraries, for example, phage display, are used to survey the molecular diversity of target cell surfaces with the aim of identifying peptide motifs that promote target cell binding or internalisation [1]. Here, an M13 phage peptide library displaying cyclic 7-mer peptides was biopanned against the luminal surface of primary cultures of rat lung alveolar epithelial cells. 'Cell associated' phage were isolated after 4 rounds of biopanning, with the peptide library repertoire contracting from 1.2×10^9 clones to a maxium of 2×10^3 clones. DNA sequencing of 'cell associated' phage clones indicated peptide sequences to be largely composed of hydrophillic amino acids with isoelectric points approximating neutrality. The most frequent phage clone bore the peptide sequence C-TSGTHPR-C (termed LTP-1) and displayed enhanced (>1000-fold) transport (versus phage control vector) across restrictive *in vitro* alveolar epithelial monolayers [2]. When the LTP-1 phage clone (LTP-1) was administered as a coarse aerosol into the airways of an isolated perfused rat lung IPRL preparation [3] the extent of phage absorption across the pulmonary epithelium was 8.6% by 120 min, some 1500-fold greater than either the insertless vector control or a library clone that displaying a control peptide sequence (C-PLLAPGI-C, termed NB-3) that was isolated from the first biopanning round. When LTP-1 phage was co-administered with a 100-fold molar excess of the synthetic LTP-1 peptide sequence (syn-LTP-1) the extent of LTP-1 phage was competitively inhibited (LTP-1 phage absorption reduced to 0.1% by 120 min, $p < 0.05$). In contrast, the synthetic NB-3 peptide (syn-NB-3) displayed no inhibitory effect (7.6% LTP-1 phage absorbed dose absorbed by 120 min, $p > 0.05$). The syn-LTP-1 peptide sequence was grafted onto the surface of an anionic PAMAM G5.5 dendrimer