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.

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A92

In vivo phage display to identify peptides that target the brain

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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.

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A93

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

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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

at a 1:1 stoichiometry to test the lung transduction functionality of the peptide using the 53 kDa dendrimer as a model macromolecular cargo. Phage peptide-dendrimer conjugates were labelled with a fluorophore and characterised by ^{1}H NMR and quantitative amino acid analysis prior to administration into the airways of the IPRL model. The extent of absorption of PAMAM G5.5 alone equalled 17 ± 6% of lung deposited dose absorbed by 90 min. G5.5 dendrimers displaying one syn-LTP-1 peptide per polymer (termed G5.5-syn-LTP-1) displayed a 1.8-fold greater extent of absorption ($p < 0.05$) cf. G5.5 alone; G5.5 dendrimer displaying one equivalent of the syn-NB3 peptide showed no evidence of enhanced absorption ($p > 0.05$). The enhanced absorption of G5.5-syn-LTP-1 absorption was competitively inhibited by co-administration of 100-fold molar excess of syn-LTP-1 peptide ($p < 0.05$) but not by syn-NB-3 peptide ($p > 0.05$), an observation consistent with the participation of a specific receptor-mediated transport mechanism. As such the LTP-1 peptide motif may serve as a platform for enhancing macromolecule absorption from the airways.

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A94
Differential transport of anionic PAMAM dendrimers across *in vitro* biological barriers

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Polyamidoamine (PAMAM) dendrimers are a class of branched polymers that have the potential to serve as drug carriers. This is primarily due to their extremely low polydispersity index, the ability to precisely control their size and charge, and the multiple functional groups that they bear on their surfaces giving the ability to conjugate a wide range of therapeutic molecules. The transport across *in vitro* biological barriers of cationic PAMAMs has been widely studied with reports often indicating high barrier permeability, although interpretation of such data in the context of cation-induced barrier toxicity is often omitted. We are investigating the intrinsic biological activity of intact stable anionic dendrimer-drug conjugates where the dendrimer moiety not only confers a backbone for attachment of multiple phamacological ligands but also offers a means to physically modulate *in vivo* tissue disposition, for example, affording access to intestinal submucosa but excluding BBB penetration. In this abstract we report the differential *in vitro* barrier permeability of a molecular weight series of anionic PAMAM dendrimers, that is, G1.5, 2935 Da; G3.5, 12,931 Da; G5.5, 52,907 Da which has supported our ongoing *in vivo* investigations. Dendrimers were fluorescently labelled and added to the apical surface of epithelial cell monolayers grown on a semi-permeable inserts (Transwell). Permeability coefficients (ρ) were determined for transport in the apical to basal direction. The epithelial models included the highly restrictive MDCK-I (TEER 5000 Ω cm^2), the moderately restrictive Caco-2 (TEER 600 Ω cm^2) and the low restrictive MDCKII (TEER 200 Ω cm^2). For CACO-2 and MDCKII an inverse relationship was evident between dendrimer transepithelial transport and dendrimer molecular size, with dendrimer ρ decreasing approx. 5-fold G1.5 ⇒ G3.5, and decreasing approx. 10-fold G1.5 ⇒ G5.5. The permeability of the cell models to dendrimer transport declined as the paracellular restrictiveness of the monolayers increased. Indeed, for MDCKI monolayers dendrimer concentrations in the basal chamber remained at all times below the limit level of detection, but could be readily enhanced by briefly adding EDTA to the media. Nevertheless, predicted (based upon LLQ) ρ for dendrimer transport across MDCKI were at least ×10–15-fold lower than in the other cell models. Significantly, even for the smallest dendrimer, that is, G1.5, the maximum predicted (based on LLQ) ρ across MDCKI was no greater than 15% of the ρ obtained for the paracellular marker F-Na. Whereas ρ for G1.5 was 51% and 56% of that for F–Na in CACO-2 and MDCKII models, respectively. Biocompatibility studies show no affect of the anionic dendrimers upon overall barrier properties. The paracellular route is the major pathway of dendrimer transport across biological barriers. Stable pharmacologically active conjugates of dendrimer – drug are an interesting experimental therapeutic with potential to provide differential tissue distribution/exclusion based upon physical characteristics.

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A95
Non-toxic, highly efficient delivery of nucleic acids into challenging cells using safectin transfection reagent

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Deliverics Ltd. has developed a novel cationic lipid-mediated transfection reagent for DNA and siRNA delivery into both easy and challenging to transfect eukaryotic cells: SAFEctin Transfection Reagent. This reagent is a water-based formulation of cationic and neutral lipids with programmed biodegradability. SAFEctin allows for the highest transfection efficiency of nucleic acids into many cell types (e.g. immortilized cells, mESC, hMSC) with the simplest-to-use and fastest procedure in the market: (i) mix SAFEctin and the nucleic acid (ii) followed by direct addition to cells, either in the presence or absence of serum and antibiotics. The formulation has been developed to have very low toxicity to cells and as such it is not necessary to remove or change culture medium following transfection. Combination of the highest/safest transfection rates on the market with the simplest to use protocol ensures optimal performance and fast results. The SAFEctin Transfection Reagent is a universal system that outperforms competitor's products in each of the three defining features any researcher seeks in this kind of product: efficacy, safety and ease of use.

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