Use of two-demensional electro-phoresis to investigate protein expression in class IIA bacteriocin-resistant Listeria Monocytogenes mutants

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PROGRAMME & ABSTRACTS

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ELISAs using the mAbs showed that *M. tuberculosis* strains antigen expression varied with stage of growth. Subtle differences in antigen recognition were observed when proteins from selected *M. tuberculosis* strains were probed with a pool of plasma from TB patients. Reactivity of individual plasma with the selected *M. tuberculosis* strains revealed different immunodominant antigens, illustrating the heterogeneous antibody response of TB patients. We have shown that protein expression patterns and antigen repertoires differ in our cohort of *M. tuberculosis* clinical isolates. This explains the heterogeneous immune response observed in TB patients and why attempts to develop a TB serodiagnostic test have been unsuccessful. Further characterisation of the antigens recognised and investigation of patterns of antigen recognition during treatment may contribute towards the development of novel markers of disease.

P018

IDENTIFICATION OF CELL SURFACE-DERIVED GLYCOCONJUGATES THAT ARE ENRICHED IN THE MEMBRANE OF PHAGOSOMES THAT CONTAIN VIRULENT MYCOBACTERIA.

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As part of their strategy for intracellular survival, mycobacteria prevent maturation of the phagosomes in which they reside inside macrophages. The molecular basis for this inhibition is only now beginning to emerge, by way of the molecular characterisation of the phagosome membrane when it encloses virulent mycobacteria. Our own work has shown that at 15 days after the phagocytic uptake of *Mycobacterium avium* by mouse bone marrow-derived macrophages, the phagosome membrane is depleted about 4-fold for cell surface-derived membrane glycoconjugates, labelled by exogalactosylation, in comparison to the membrane of early endosomes with which it continues to interact. Here we report first results to complement these quantitative results at a molecular level by trying to identify specific cell surface-derived membrane glycoconjugates that remain associated with the phagosomal membrane in spite of the general depletion observed. For this purpose, bone marrow-derived mouse macrophages were infected with virulent *Mycobacterium avium*. After about 7 days post-infection, the macrophage plasma membrane was labelled enzymatically with ^{3}H-galactose, followed by endocytosis to redistribute this surface label to membranes of endocytic organelles, including mycobacteria-containing phagosomes. As a control for cell-surface membrane glycoconjugates, the plasma membrane of infected cells was labelled with ^{14}C-galactose without further endocytosis. ^{14}C-labelled plasma membrane and ^{3}H-labelled phagosomes were isolated on sucrose density-gradients and analysed by 2-dimensional electrophoresis. First results indicated that proteins in the 50-55kD range are retained in the phagosomal membrane in an enriched fashion.

P019

USE OF TWO-DIMENSIONAL ELECTRO-PHORESIS TO INVESTIGATE PROTEIN EXPRESSION IN CLASS IIA BACTERIOCIN-RESISTANT *LISTERIA MONOCYTOGENES* MUTANTS.

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Global protein expression of several of *Listeria monocytogenes* mutants with high levels of resistance to an various class IIa bacteriocins, which have biopreservative potential, were assessed using two dimensional (2-D) electrophoresis. A rapid and reliable total protein extraction procedure was optimised for analysis of gene expression in *L. monocytogenes*. The proteomes of several food isolates were compared with the profile of *L. monocytogenes* EGDe, the genome sequence of which has recently been released. In comparing 2-D gels of proteins extracted from wild-type strains and corresponding resistant mutants, a single spot was found to be consistently missing from the latter. In an attempt to determine the cellular location of this protein, a modified compartmentalisation procedure was utilised. The single protein spot consistently missing from resistant mutants was located in the membrane fraction. In order to provide sufficient resolution for identification of this spot, a zoom-gel of the corresponding pI range was run. This showed the single protein to be a cluster of six protein spots in all wild type strains analysed, however the entire cluster was found to be absent from all the resistant mutants. Preliminary results indicate that the cluster is composed of a single protein with high homology to the enzyme IIAB2 component of the mannose phosphotransferase system (PTS). These results corroborate the hypothesis that the mannose PTS in *L. monocytogenes* is involved in generation of high levels of resistance to class IIa bacteriocins (see also abstract by Gravesen *et al.*).

P020

THE MOLECULAR EPIDEMIOLOGY OF BEIJING-LIKE *M. TUBERCULOSIS* ISOLATES IN A HIGH INCIDENCE COMMUNITY.

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Setting: A retrospective study in an urban setting with a high tuberculosis incidence.
Objective: To study the molecular epidemiology in of a highly prevalent *M. tuberculosis* strain family, F29, which shows genotype similarities with the Beijing-strain group identified globally.