Project objectives for the period

Studying the systems biology of intracellular bacterial infections within the host cell, as opposed to axenic culture, is imperative. However, since pathogens interfere with host-cell proteins and host cells respond to bacterial infection, both sides have to be considered to obtain appropriate data sets relevant for anti-bacterial intervention. The overall aims in this first period are to develop initial models, establish the various assays for RNAi-based screening of infected cells, and collect the first data sets. The deliverables for this period are as follows:

1.1 – Initial Model

- Manually develop an initial crude model of phagocytosis following the traditional knowledge-based modelling paradigm. Validate this model with measured data; conduct sensitivity analysis to suggest new experiments.
- Identify problems/weaknesses of automated modelling (AM) methods in the context of modelling phagocytosis and similar problems from systems biology.
- Initial model with validation results, list of requirements for AM methods in light of systems biology.

1.2 – Report on modelling phagocytosis for website

2.1 – High-throughput Imaging assay

- Generation of high throughout phenotypic cell-based assays to quantitatively assess bacteria entry, phagosome trafficking and intracellular bacteria clearance suitable for high-content screening using genomic RNAi libraries in murine macrophages/cell lines.

2.2 – Predictive models of phagosome maturation

- Development and adaptation of image analysis software to extract quantitative parameters from images produced from the aforementioned assays.

2.3 – Data array/ phagosome maturation

- Generation of quantitative data suitable to theoretical analysis and development of predictive models of phagosome maturation.

2.4 – Report on assay development and phagosome characterisation for website

3.1 – Focused data array / uptake

- A data array comparing the involvement of kinases and small G proteins in Salmonella and mycobacteria uptake.

3.2 – Report about RNAi screens for uptake, for website

4.1 – Focused data array / pathogen survival

- Data array comparing involvement of Rab modifying proteins (Rab GTPases, Rab GAPs) in Salmonella and M. tuberculosis intracellular survival in primary human macrophages (Partners 2 & 3).

4.2 – Report about RNAi screens for maturation for website

5.1 – Data array/pathogen genes

- List of drug-target-candidate pathogen genes involved in modulation of phagocytosis by host cells.

5.2 – Report on manipulation of phagosome maturation by pathogens for website

Initially we will construct models of phagocytosis based on current knowledge, and available experimental data. This will allow us to refine existing methods and provide a starting point for predicting interference points that would prevent phagosome-lysosome fusion. In parallel, we will optimise protocols and develop assays amenable to high throughput RNAi screening to identify genes involved in the trafficking of the intracellular pathogens mycobacteria and Salmonella. We will establish protocols to characterise the bacteria-containing compartments of phagocytic cells. Image analysis will be used for co-localisation/co-compartmentalisation studies, thereby locating the bacteria in the different types of phagosomes and characterising the host cell markers associated with those compartments. Imaging experiments will also be done using siRNA or chemical compounds shown to modulate bacterial growth in the earlier screens. As well as characterising the phagocytic pathway for intracellular pathogens and establishing potential interaction points from the host cell side, we will also investigate what bacterial components contribute to this process. In particular mycobacterial proteins will be sought that mediate the interactions with macrophage intracellular molecules and prevent phagosome-lysosome fusion, thereby promoting bacterial survival. We will further characterise existing bacterial mutants that are altered in their ability to survive intracellularly and compare their survival in different cell types.

As the project progresses the data collected from the experimental work will then be used in the models. Are the predicted end compartments consistent with those observed experimentally? Can the interference points identified in the experimental analysis be predicted by the models? Can this information be used to improve the models, and can the models predict other intervention points that could be targeted chemically or genetically? The ultimate aim of this project is to suggest new interventions that will promote clearance of intracellular bacteria by phagocytic cells, and so boost innate immunity.

The project was reviewed by our external advisory group at our mid-term meeting in Dresden. Each work-package presented their progress to date and future plans. Their report is attached at the end of this document. Their major recommendations and our response are summarised here and given in detail in the body of the report:

- Encourage focused screenings (kinome, phosphotome)

  this will be taken forward by WP4, who have already made progress in this area.
- Incorporate biochemical analyses (PIP)

  this will be incorporated in the model being developed in WP1 in collaboration with the other partners.
- Extended lab visits for modellers to focus questions of the experimentalists and to learn more about the biology

  we have already begun to do this, as detailed in the management section of the report, and will continue to do this as budget allows.
- More sharing of protocols (Wiki page)

  we have already updated this so all partners can see the protocols currently in use. It is planned to make agreed versions of protocols available on our public website at a later date. This report will be placed on the PHAGOSYS public website as fulfilment of the report deliverables.