Sub-project 3

- How does triggering alternate or multiple PRRs affect the outcome?
- What is the impact of endogenous signalling pathways on pathogen sensing?
The experimental system

Mouse bone marrow-derived dendritic cells
8d culture with GM-CSF (X63 sn)

• RNA: qRT-PCR, microarray
• Supernatant: ELISA
• Protein: phosphoP analysis

Analysis over time
4 treatments, time course over 4 hrs with samples taken every 30 mins.
- Triplicates at 0 hrs, 2 hrs and 4 hrs.
- Total of 60 microarrays.
- Previously characterised genes show consistent patterns
- Total of 884 genes altered at 4hr in at least one group

Total number of regulated genes: 884
Modelling microarray data

Rate of change of expression of a gene

$$\frac{dx_i(t)}{dt} = \beta_i + S_i f_i(t) - \alpha_i x_i(t)$$

Transcription factor activity

Basal rate

Sensitivity

Decay rate
Analysis of Time Course Data

Graphs showing gene expression over time for IgG1, Jgd, LPS + IgG1, and LPS + Jgd.

ODE Model of Gene Expression

Error Function

A putative list of genes whose expression is driven by the same transcriptional activity.
Two main groups

\[ Jgd + LPS \]

\[ LPS \]
Validation

- Model predicts genes that are regulated in a similar fashion.
- Model estimates degradation rates.
- Model can compare genes across treatments.
- Validate predictions by promoter analysis, chromatin immunoprecipitation and use of small molecule inhibitors.
Integration of Sub-projects

1. Glycans Expression
   - Machine Learning
   - Bayesian Nets
   - Ordinary Differential Equations

2. Phagocytic Response
   - Uptake
   - Brane Calculus
   - Partial Differential Equations

3. Transcriptional Response
   - Machine Learning
   - Ordinary Differential Equations

CISBIC
Triggering of cytokine expression by \textit{C. jejuni} mutants

WT: Wild type \textit{C. jejuni}

cj1417: side branch of capsule missing
cj1439: acapsular

\begin{align*}
\text{TNF}_\alpha \text{ expression in DCs after infection with } & \text{C. jejuni} \\
\text{IL-6 expression in DCs after infection with } & \text{C. jejuni}
\end{align*}
Cytokine expression in some mutants is similar to WT *C. jejuni*

**IL-6 expression in DCs after infection with C. jejuni**

**WT**

**PglB**

MOI: Multi-Organism Index

**WT: wild type**

**PglB:** no N-linked glycosylation

**FlaA:** no flagella
Notch signalling can enhance IL-10 production from C. jejuni

IL-10 expression in the presence and absence of Jgd

MOI=100  MOI=20  MOI=10  MOI=1  none

fold change (arbitrary units)

- IgG1
- Jgd
Summary

- Notch modulates TLR signalling and vice versa in DCs
- Model has generated some predictions of genes co-regulated with IL-10
- Work on sub-project integration has started, investigating the cytokine response of DCs to different *C. jejuni* mutants.
Achievements

- Detailed biological exploration of the system including
  - Time course microarray analysis
  - Measurement of degradation rates
  - Investigation of the role of a number of signalling pathway components.
- Modelling of global transcriptional response
Future plans

- Predictions generate groups of genes that are similarly regulated.
- Validation of those predictions will initially focus on promoter analysis, chromatin immunoprecipitation, measurement of degradation rates and use of small molecule inhibitors.
- Integration with sub-project 1 will continue. Areas to investigate include the role of TLR4 and LOS in the innate immune response to C. jejuni and whether different WT strains elicit different levels of cytokine response.
- Initial experiments for integration with sub-project 2 are planned.
Thank you