Understanding phagocytic receptor localisation and signalling

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Analysis of early signaling following phagocytic receptor engagement
Aims - Biological sub-project 2

Experimental work divided into two parts:

A - Fc receptor dynamics during early stages of phagocytosis - (with Jeroen Van Zon / Martin Howard)

B - Identification of molecules involved in phagocytosis signalling networks
Aims - Biological sub-project 2

A - Fc receptor dynamics during early stages of phagocytosis -
(with Jeroen Van Zon / Martin Howard)

Generation of Fc receptor mutants (GT)

Confocal microscopy of live/fixed cells undergoing phagocytosis under various conditions (GT)

Generation of model for Fc receptor distribution (JVZ/MH)
A. Fc receptor dynamics during early stages of phagocytosis

Experimental work -

Use of confocal microscopy to analyse:

Diffusion of receptors across membrane

Movement of Fc receptors in and around phagocytic cups during phagocytosis (time-courses) -

done for a variety of mutants:

wild type, signalling mutant, deleted cytoplasmic tail…

and particle sizes
A. Fc receptor dynamics during early stages of phagocytosis

Example images

Beads-IgG  |  Actin  |  FcγRIIa  |  Merge

WT FcγRIIa 6 min

signalling mutant FcγRIIa 6 min
A. Fc receptor dynamics during early stages of phagocytosis

Observations:

Fc receptor (but not actin) cups are still able to form in mutants:

- medium particles have uneven/misshapen Fc receptor cups
- small particle mutant Fc receptor cups look similar to wild type

Very large particles begin to form cups but do not progress

Collected time-course data for Fc receptor and actin localisation
A. Fc receptor dynamics during early stages of phagocytosis - Modelling

   Membrane shape model

   Constructed model coupling membrane/cytoskeletal dynamics to receptor diffusion and signalling

   Completely determined by bending modulus and cortical tension

   Model correctly predicts shape of cup
A. Fc receptor dynamics during early stages of phagocytosis - Modelling

Phagocytic cup shapes predicted by membrane model

![Graph showing early and late phagocytic cups](image)

Force required to make a cup of certain size can be calculated
A. Fc receptor dynamics during early stages of phagocytosis - Modelling

Diffusion model

Fc receptors bind antibody on particle and stimulate actin polymerisation

Local actin polymerisation provides force for cup formation
A. Fc receptor dynamics during early stages of phagocytosis

- Future plans

  • Further analysis of microscopy data, spatio-temporal localisation of Fc receptor and actin. Add to model
  
  • Incorporate more complicated signalling dynamics, get more realistic results