Secretory cytotoxic granule maturation and exocytosis require the effector protein hMunc13-4

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Research Aim

Investigate the role of Munc13-4 in maturation and secretion of lytic granules
Regulated secretion from CTLs

Activated CTL polarisation

- T-cell Receptor binding
- Micro tubule organising centre polarisation

Small GTPase Rab27a

- Griscelli syndrome type 2 (GS2)
  - Lytic granules fail to dock after moving to the immunological synapse in activated CTLs

- Small GTPase; regulators of vesicle formation, transport and fusion
Munc13-4

- Familial Haemophagocytic Lymphohistiocytosis type 3 (FHL3)
  - Impaired lytic granule exocytosis

- Member of Munc13 family, involved in vesicle tethering

Putative hMUNC13-4 domain organisation

Rab27a and Munc13-4

- Rab27a and Munc13-4 interact directly regulating secretion in Mast cells (Neeft et al., 2005)

Mechanism of regulation unknown
Research Aim

Investigate the role of Munc13-4 in maturation and secretion of lytic granules
Cytotoxic granules do not colocalise with Rab27a or hMunc13-4
Rab27a localises to late endosomes

Figure 2a
GFP-Rab11
DsRed-Rab27a

Figure 2b
GFP-Rab7
DsRed-Rab27a
hMunc13-4 localises to recycling endosomes and Rab27a

Figure 3a

GFP-Rab11
hMunc13-4–DsRed

Figure 3b

GFP-Rab27a
hMunc13-4–DsRed
hMunc13-4 directs late and recycling endosome assembly

GFP-Rab11
DsRed-Rab27a

No overexpressed hMunc13-4

Figure 2a

Overexpressed, unlabeled hMunc13-4

Figure 3d
Recap

• Rab27a localises only to late endosomes (Rab7)

• Rab11 and Rab27a compartments colocalise in the presence of hMunc13-4

• In FHL3 cells, hMunc13-4 introduction induces Rab11 and Rab27a overlap
MHD regions of hMunc13-4 facilitates recycling endosomes localisation

Figure 5b
hMunc13-4 assembly function independent of Rab27a

Figure 3a

Figure 5c
Rab27a and Rab11 structures localise at immunological synapse

Figure 7a
The same as in resting state.. But in resting state not localised to synapse

No overexpressed hMunc13-4

Overexpressed, unlabeled hMunc13-4

Figure 2a

Figure 3d
Cytotoxic granules localise with Rab11 & Rab27a vesicles at cell contact sites

Figure 1

Anti-perforin
hMunc13-4–DsRed

Anti-perforin
hMunc13-4–DsRed

Target

CTL

Figure 7b
Rab11$^+$ endosomes and cytotoxic granules recruit to the synapse

- hMunc13-4 deficient
- Endogenous hMunc13-4
- Overexpressed hMunc13-4
Movie S6

GFP-Rab11 and Anti-perforin localise to the cell-cell contact site
Conclusions

• hMunc13-4 facilitates assembly of recycling & late endosomes, independent of Rab27a or CTL activation

• hMunc13-4 is required for fusion of exocytic endosomes with cytotoxic granules at the immunological synapse
Conclusions

CTL

Lytic Granules

Rab11

hMunc

Rab27a
Conclusions

Cell contact site

Lytic Granules

Rab11

Rab27a

hMunc

Lethal hit

Cell-Cell contact site

Target Cell
Discussion

• What about early endosome association of hMunc13-4?

• Everything was done with overexpression → effect on Rab localisation?
Discussion

• Recycling and late endosome “fusion” is shown by colocalisation of Rab11 with Rab27a

![Figure 3c](image)

• Can you say this is a fusion event? They don’t show that Rab11 colocalises with Rab7 in the presence of Munc13-4
What is the function of Rab27a binding to Munc13-4?

• Munc13-4 can assemble late and recycling endosomes, but where does Rab27a fit in the picture?
Figure 3d
Cell activation does not regulate hMunc13-4 levels

- Similar overlap was found in activated and non-activated T-cells.
Only active Rab27a binds to (N-terminal of) hMunc13-4
Figure 6b: Rab27a interacting region of hMunc13-4