

# Tuberculosis Toxin Blocking Phagosome Maturation Inhibits a Novel $Ca^{2+}$ /Calmodulin-PI3K hVPS34 Cascade

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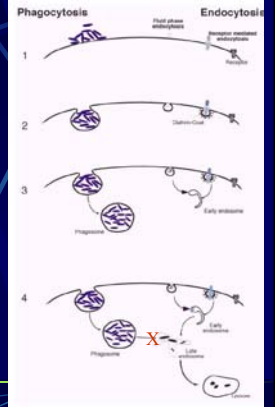
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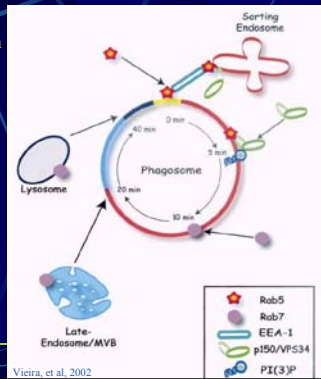
*Mycobacterium tuberculosis* is an intracellular pathogen that depends on its ability to block maturation of phagosomes to survive inside macrophages

Normal phagosome maturation leads to the fusion with lysosomes ending in pathogen death

How does *M. tuberculosis* inhibit phagosome maturation?

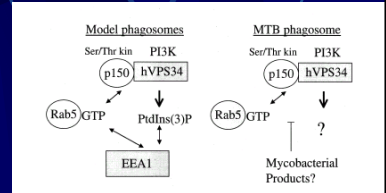


- Series of fusion steps with endocytic vesicles
- Rab5, early endosome antigen 1 (EEA1), p150 and PI(3)P complex required prior to endosomal SNARE function
- EEA1 provides a functional link between Rabs and SNAREs



What was already known about *M. tuberculosis* block of phagosome maturation?

- Mycobacterium tuberculosis* inhibits the recruitment of EEA1 to the maturing phagosome



Fratti, et al, 2000

- Mycobacterium tuberculosis* inhibits cytosolic  $Ca^{2+}$  rise

*Mycobacterium tuberculosis*

Cytosolic rise in  $Ca^{2+}$

Phagosome maturation

EEA1 accumulation on phagosome

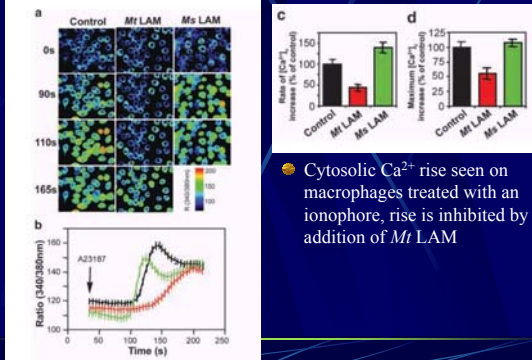
*Mtb*

$Ca^{2+}$  increase

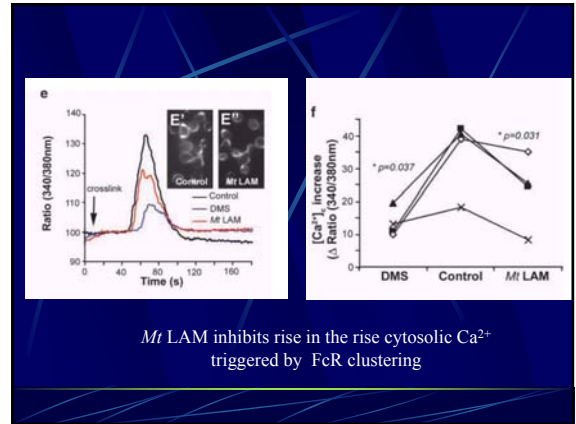
EEA1 accumulation on phagosome

Hypothesis: The effect of *M. tuberculosis* on  $Ca^{2+}$  fluxes leads to an inhibition of EEA1 accumulation on the phagosome subsequently blocking phagosome maturation

### Mt Lam inhibits cytosolic rise in Ca<sup>2+</sup>



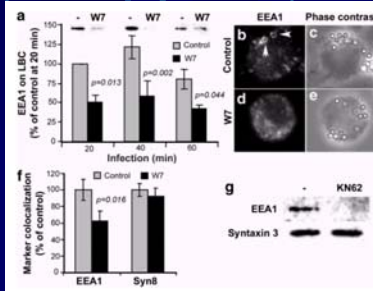
● Cytosolic Ca<sup>2+</sup> rise seen on macrophages treated with an ionophore, rise is inhibited by addition of Mt LAM



Mt LAM inhibits rise in the rise cytosolic Ca<sup>2+</sup> triggered by FcR clustering

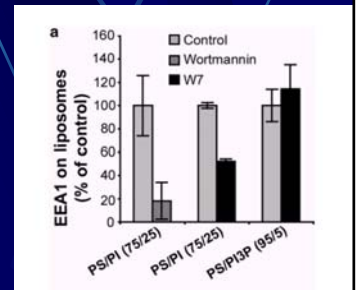
### Ca<sup>2+</sup>/calmodulin required for recruitment of EEA1 to phagosome

● EEA1 accumulation on LBC's disrupted by addition of W7, a calmodulin inhibitor as well as KN62 an inhibitor of the Ca<sup>2+</sup>/calmodulin effector CaMKII

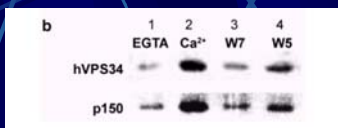


### Ca<sup>2+</sup>/calmodulin important in recruitment of PI3-kinase

Addition of W7 only disrupts EEA1 accumulation on phagosome in the absence of pre-existing PI



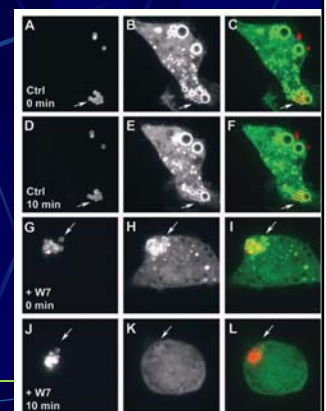
### Ca<sup>2+</sup>/ calmodulin bind to type III PI3-kinase hVPS34



Ca<sup>2+</sup> binds to the PI3-kinase hVPS34 only in the absence of W7

### PI3-kinase associates with phagosomes only in the presence of Ca<sup>2+</sup>/ calmodulin

PI3-kinase localization to the phagosomal membrane inhibited by addition of W7



## Conclusions

- *Mt* Lam inhibits intracellular rise in  $\text{Ca}^{2+}$
- Inhibition of  $\text{Ca}^{2+}$  /calmoduin prevents recruitment of PI3-kinase to the phagosome
- Inhibition of PI3-kinase prevents EEA1 recruitment
- Lack of EEA1 on phagosome blocks further maturation

## *Mt* LAM inhibits a novel signaling pathway in phagosome maturation

