How EsxH Controls Host Cellular Responses to *Mycobacterium tuberculosis*

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Mtb survives inside macrophages

Mtb impairs lysosomal trafficking and antigen presentation.

Mtb persists despite robust CD4+ T cell responses.

A effective TB vaccine will have to overcome Mtb’s immune evasion strategies.

Mtb virulence factor, EsxG-EsxH, is critical for immune evasion. EsxG-EsxH impairs both lysosomal trafficking and antigen presentation.
EsxG-EsxH inhibits the host factor HRS

- EsxG-EsxH is critical for virulence.
- EsxG-EsxH from Mtb binds HRS (ESCRT-0).
- EsxG-EsxH from Msmeq does not bind HRS.
- By targeting HRS, EsxG-EsxH inhibits lysosomal trafficking and MHC II antigen presentation.

EsxH impairs antigen presentation

Dendritic cells (DCs) were infected with WT, ΔesxH, or ΔesxH::esxH

Infected DCs were co-cultured with CD4⁺ T cells that recognize Mtb Antigen 85B peptide.

IFN-γ release by T cells reflects antigen presentation.

EsxH inhibits antigen presentation by targeting HRS/ESCRT.

ESCRT is composed of sub-complexes that traffic receptors for lysosomal degradation

ESCRT (Endosomal Sorting Complexes Required for Transport)

ESCRT-0; HRS
ESCRT-III; CHMPs (CHMP1A, CHMP1B, CHMP4A, CHMP4B)

Vingtdeux V et al., Frontiers in Physiology 2012
Outstanding Questions…

➢ How does EsxH alters HRS/ESCRT function?

➢ Does it influence downstream ESCRT molecules?

➢ Does EsxH alter other aspects of ESCRT activity like exosome formation?

➢ Does it influence antigen transfer to uninfected cells by altering exosomes?
EsxH prevents the recruitment of HRS to Mtb phagosomes

Immuno-EM of Hrs in Mtb infected BMDM

Conclusions: EsxH causes HRS to redistribute to the cytosol. EsxH inhibits HRS recruitment to Mtb phagosomes.
EsxH antagonizes ESCRT-III recruitment

Co-localization of CHMP4B with Mtb

BMDMs, 3 hpi

P=0.017
EsxH impairs ESCRT-III recruitment

**Conclusion:** EsxH antagonizes ESCRT-0 (HRS) and ESCRT-III recruitment to Mtb phagosomes.
ESCRT is involved in exosomes and microvesicles production

Hypothesis:
EsxG-EsxH alters exosome production by targeting ESCRT, thereby impairing antigen transfer and/or altering cytokine responses.
Does EsxH impair exosome formation?

DCs infected with Mtb, ΔesxH, or ΔesxH::esxH

Exosome

Uninfected | WT Mtb | ΔesxH
---|---|---
LAMP1
Tsg101
CD63
Antigen85B
β-Actin

Cell Lysate

Uninfected | WT Mtb | ΔesxH

Conclusion
EsxH impairs exosome secretion, consistent with its ability to inhibit ESCRT.
Does EsxH qualitatively alter exosomes?

Exosome Analysis: Nanosight, Size distribution by number

Uninfected

WT Mtb

ΔesxH

ΔesxH::esxH

Shifted towards smaller size

Conclusion: EsxH impairs exosome secretion and changes their size distribution.
ΔesxH-infected DCs produce exosomes with enhanced ability to activate CD4+ T cells

In progress: How does EsxH affect the composition of exosomes? What is the consequence of altered exosome production? Does it impair antigen transfer? Does it contribute to altered cytokine responses?
Conclusions

- EsxH antagonizes HRS and ESCRT-III recruitment to Mtb phagosomes.
- EsxH impairs exosomes formation.
- EsxH plays a central role in Mtb evasion of innate and adaptive immunity.
- The ability of EsxH to block exosome production may contribute to immune evasion.
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