CHLAMYDIA TRACHOMATIS CAUSES MITOCHONDRIAL DAMAGE IN KERATINOCYTES

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INTRODUCTION

Chlamydia trachomatis

- Three biovars:
  - Trachoma
  - Genital discharge (GD) disease
  - Lymphogranuloma venereum (LGV)

  } Both STI biovars invade epithelium but only LGV strains enter underlying tissues

- Unique biphasic replication cycle within a membrane bound inclusion in the host cell
  - Elementary body (EB) – infectious metabolically inert extracellular form
  - Reticulate body (RB) – non-infectious metabolically active intracellular form

- Prevalence in South Africa:
  - LGV (ulcers) – 14% male, 19% females
  - GDD – 16% male urethritis, 11% female cervicitis

- Possesses genes encoding proteins with significant homology to the large cytotoxins A and B which are produced by *Clostridium difficile*
- GD isolates express the active portion with glucosyltransferase activity (CT166), but LGV isolates do not
- This is a preformed cytotoxin within the EB which is present on initial infection
- The large clostridial cytotoxins cause disassembly of actin microfilaments and mitochondrial damage
- GD but not LGV isolates cause disassembly of actin microfilaments and cell rounding in infected cells, but the effect on mitochondria has not yet been studied

METHODS

Cells
- McCoy cell line (mouse fibroblasts) – used for propagation of chlamydia
- HaCaT cell line (human keratinocytes) – used for experiments

Chlamydia trachomatis
- LGV biovar: serovar L2 strain 434/Bu
- GD biovar: serovar E clinical isolate

Transmission electron microscopy (TEM)
1. Cells grown in 24-well plates with Thermanox coverslips were infected (MOI=0.25) with *C. trachomatis* and incubated at 37 °C
2. At 1, 3, 9, 18, 24, 36 and 48 h post-infection, cells were fixed with 2% glutaraldehyde and processed for TEM
3. Ultra-thin sections were viewed on a JEOL-1011 or JEOL-1010 TEM and photographed
4. The diameter of 15 mitochondria in the mock infected control and in serovar E and L2 infected cells at 1,36 and 48 hours post infection was measured

Methyl thiazolyl tetrazolium (MTT) assay
1. Cells grown in 96-well plates were infected (MOI=0.25) and incubated at 37 °C
2. After 1, 3, 9, 18, 24, 36 and 48 h post-infection an MTT assay was performed
3. O.D. was measured at 570 nm with reference at 630 nm

$$\text{Percentage cytotoxicity} = \frac{\text{O.D. chlamydia exposed cells}}{\text{O.D. chlamydia unexposed cells}} \times 100$$

Statistical analyses
- Mean mitochondrial diameters were compared using a One Way Analysis of Variance with Tamhane's T2 post-test
- Mean percentage mitochondrial activity was compared using a two-tailed paired T test
- $P \leq 0.05$
- SPSS version 23 was used

REFERENCES

Belland RJ, Scidmore MA, Crane DD, et al. 2001. *PNAS* **98**:13984-9
Carlson JH, Hughes S, Hogan D, et al. 2004. *Infect Immun* **72**:7063-72
Joubert BC, Sturm AW. 2011. *J Med Micro* **60**:1565-9
Matarrese P, Falzano L, Fabbri A et al. 2007. *J Biol Chem*. **282**:9029-41
Wakabayashi T. 1999. *Acta biochimica Polonica*. **46**:223-37
Ward ME. 1983. *Br Med Bull* **39**:109-15

RESULTS

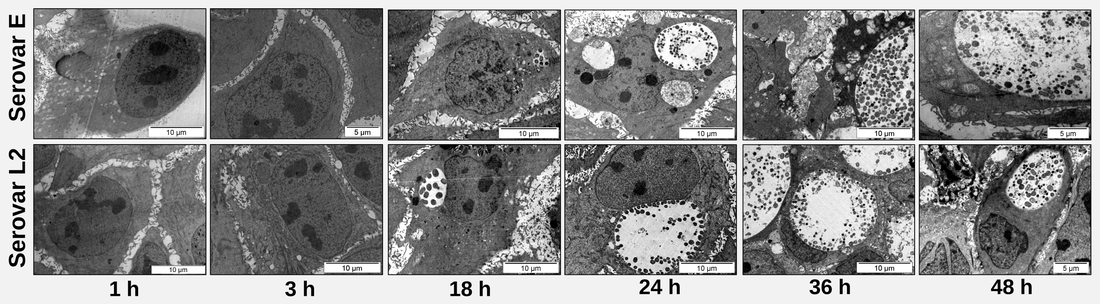

Fig. 1. Electron micrographs giving an overview of the HaCaT cell during the *C. trachomatis* replication cycle.

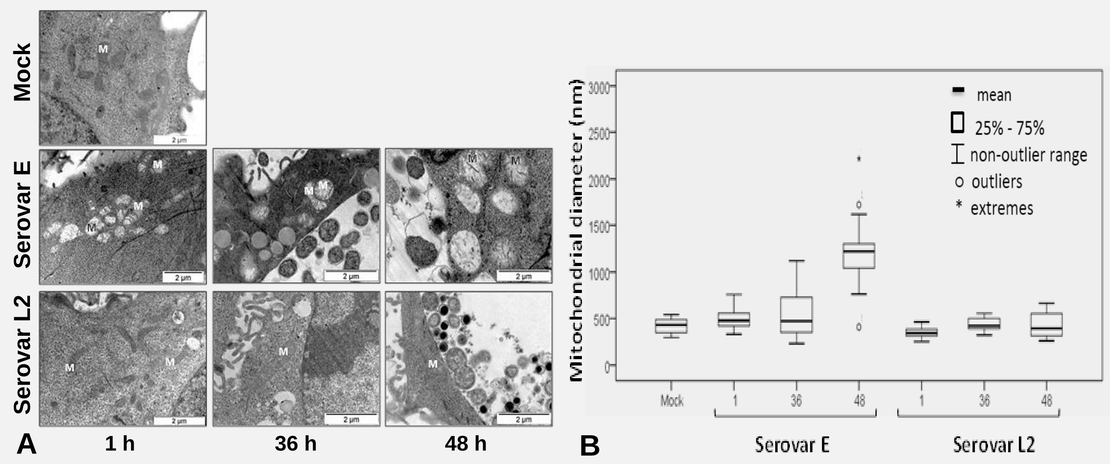

Fig. 2. Comparison of mitochondrial morphology and diameter in *C.trachomatis* infected HaCaT cells at 1, 36 and 48 hours post infection versus the mock infected control.
(A) Electron micrographs indicating the morphology of mitochondria (M) in chlamydia-infected HaCaT cells and the mock infected control at selected time points. (B) Mitochondrial diameter in chlamydia-infected HaCaT cells and the mock infected control at selected times post infection. Mean mitochondrial diameter in serovar E infected cells at 48 h post infection was significantly larger when compared to all other groups ($P \leq 0.001$).

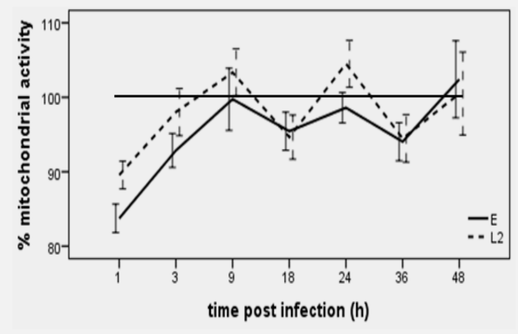

Fig. 3. Percentage mitochondrial activity in *C. trachomatis* infected HaCaT cell monolayers at 37° C.
Mitochondrial activity was quantified using the MTT assay and expressed as a percentage of the mitochondrial activity of mock infected monolayers at the corresponding time post infection. Mitochondrial activity of mock infected cells was considered to be 100%. Error bars represent ± 1 standard error of the mean.

DISCUSSION AND CONCLUSIONS

The *C. trachomatis* replication cycle and the structure of the EB, RB and inclusion is the same in HaCaT keratinocytes at 37 ° C as compared to other cell lines which are not the wild type host.

The GD isolate but not the LGV strain caused changes in mitochondrial morphology 1 hour after infection. Normal mitochondrial morphology was restored by 3 hours post infection, but the altered mitochondrial morphology returned at 36 hours post infection together with swelling at 48 hours post infection. This correlates with the presence of EB in the cell.

EB of GD chlamydia are known to contain a preformed cytotoxin containing the active site of the large clostridial cytotoxins with glucosyltransferase activity. These toxins cause disassembly of actin microfilaments and mitochondrial damage.

Further research is needed in order to fully understand the relationship between *C. trachomatis* and mitochondria.

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