CHLAMYDIA TRACHOMATIS CAUSES MITOCHONDRIAL DAMAGE IN KERATINOCYTES

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INTRODUCTION
Chlamydia trachomatis

- Three biovars: Trachoma, Genital discharge (GD), and Lymphogranuloma venereum (LGV)
- Unique biphasic replication cycle within a membrane bound inclusion in the host cell
- Prevalence in South Africa: 14% male, 19% females
- GD isolates express the active portion with glucosyltransferase activity (CT166), but LGV isolates do not
- The LGV strain enters normal cells and infects the inner layers of the host

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 434/8u
- GD biovar: serovar E clinical isolate

Transmission electron microscopy (TEM)
1. Cells grown in 24-well plates with Thermaxon 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 3.6 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 to 630 nm

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 ≤ 0.05
- SPSS version 23 was used

RESULTS

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 cytoplasmic 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.

REFERENCES

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