

New Insight in the Pathogenesis of LGV

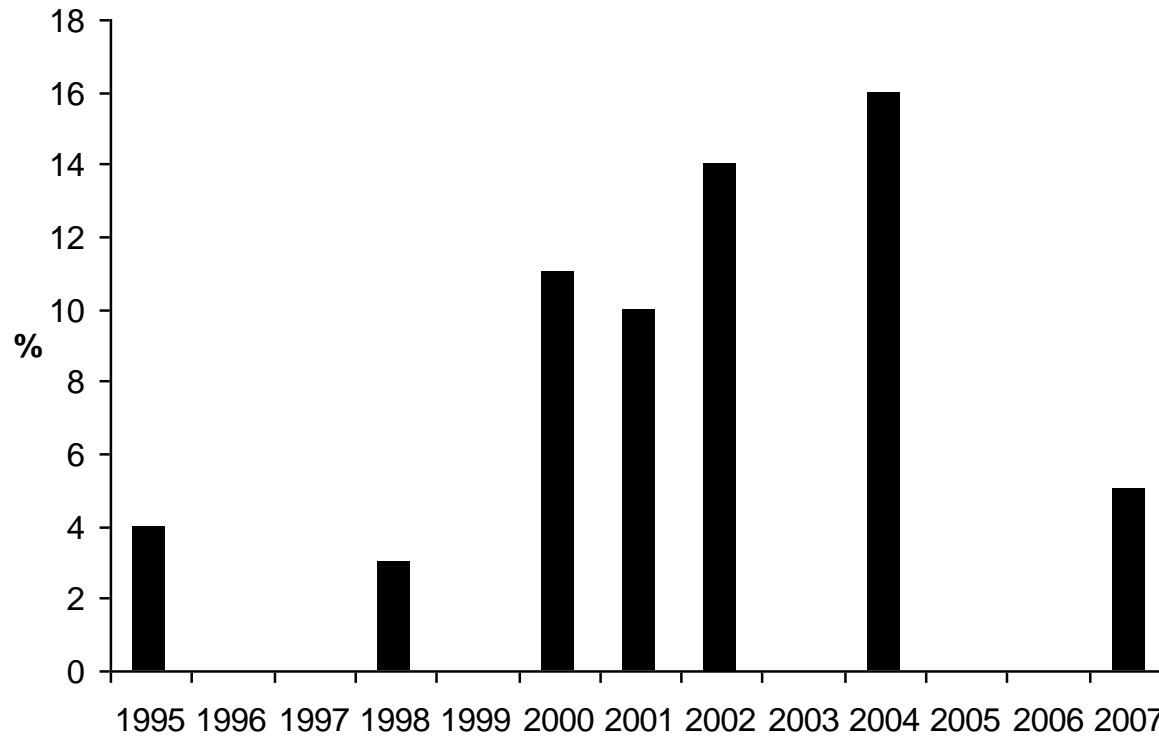
Wim Sturm

University of KwaZulu-Natal

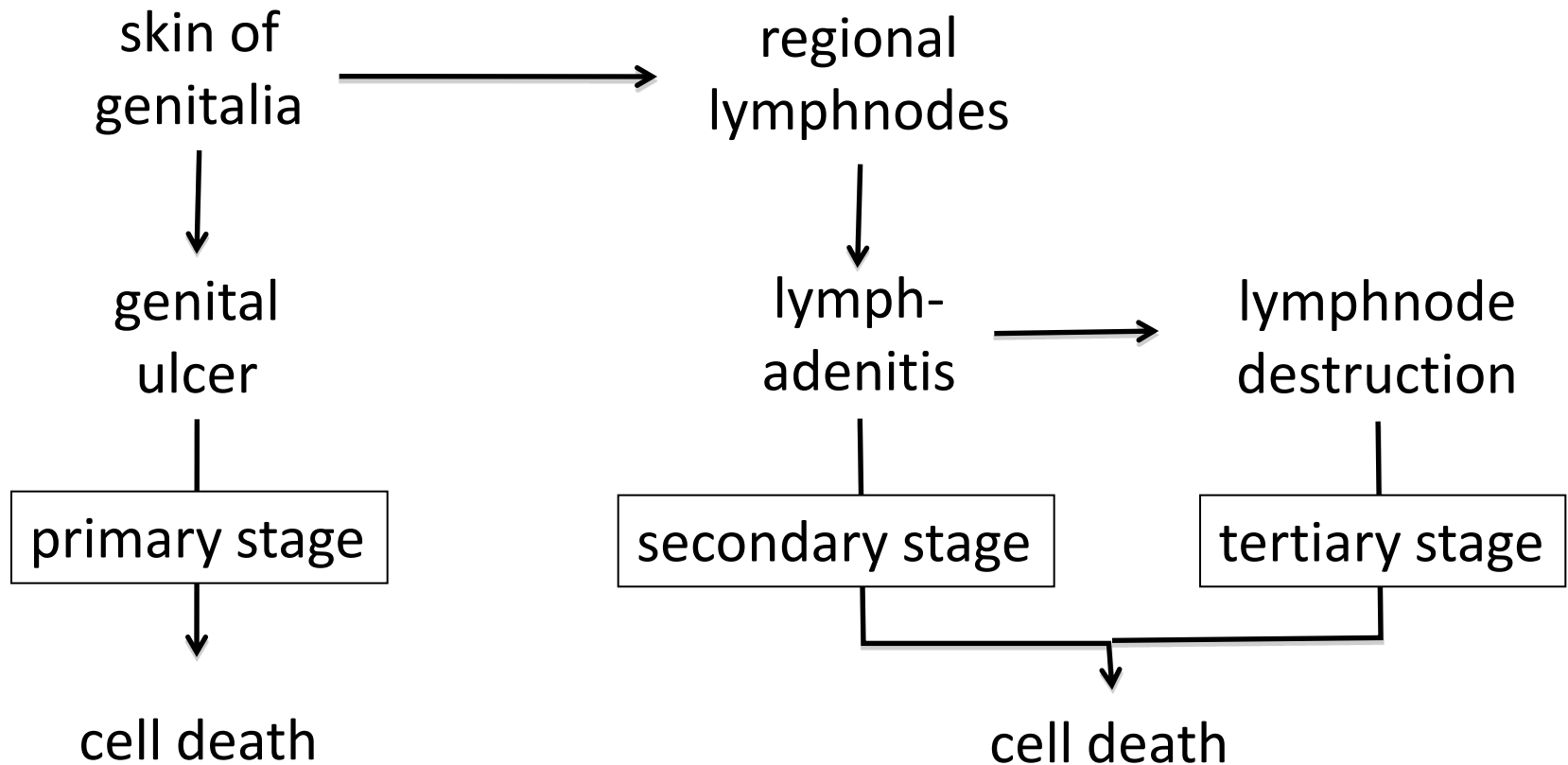
Chlamydia trachomatis

- Obligate intracellular human pathogen
- Causes trachoma, urogenital disease and LGV
- 2 biovars
 - based on differences in disease presentation + in vitro cell growth characteristics
- 18 serovars
 - based on immunological cross-reactivity of MOMP
- Oculogenital (OG) biovar
 - urogenital disease and conjunctivitis (D through K, Da, and Ia)
 - ocular trachoma (A, B, Ba, and C)
- Lymphogranuloma venereum (LGV) biovar
 - LGV (L1, L2, L2a, and L3)

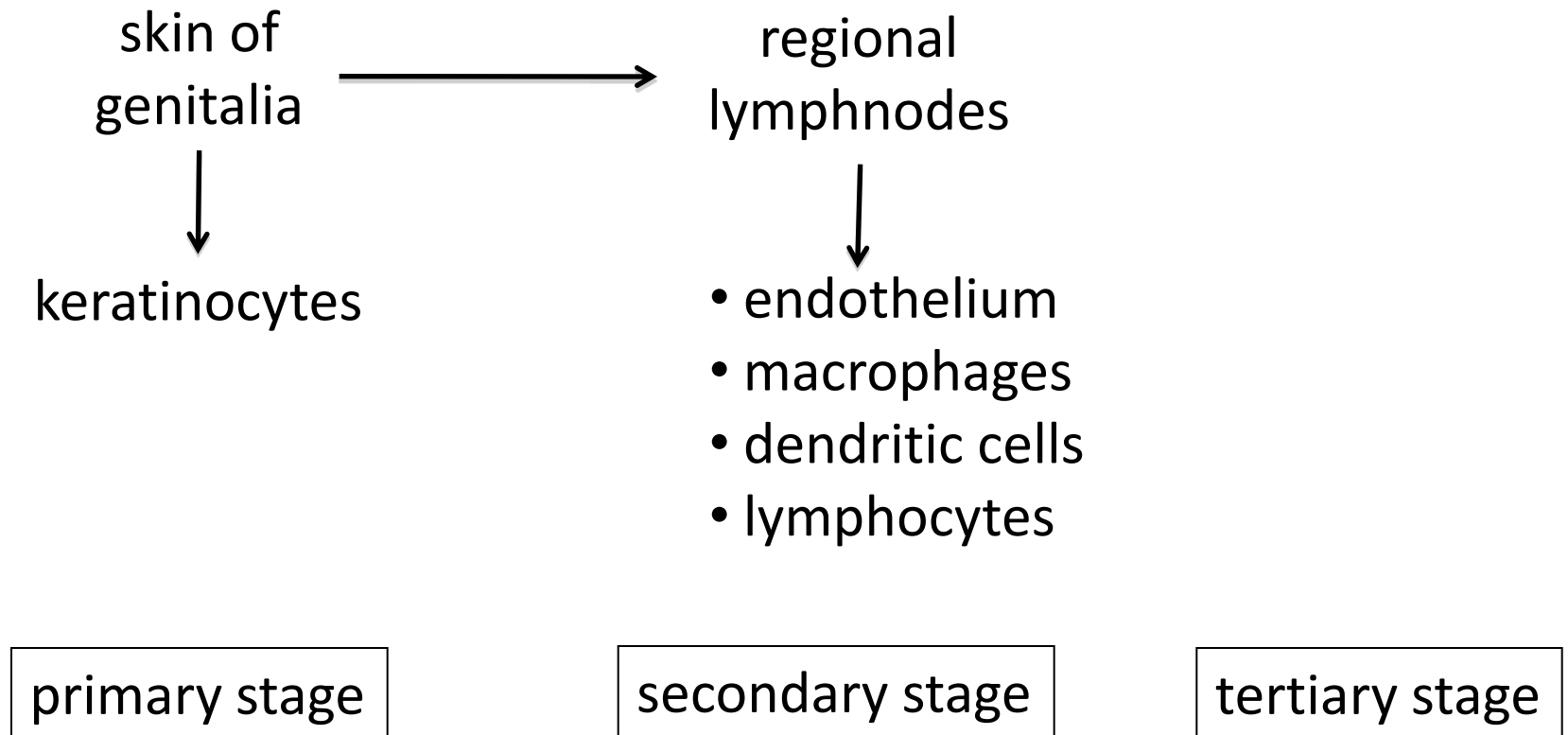
Prevalence of LGV in patients with genital ulcer disease in Durban



Lymphogranuloma venereum



Lymphogranuloma venereum



Chlamydia and host-cells

early phase → inhibition of apoptosis



late phase → apo-necrosis

Growth of *C. trachomatis* in cells

- HeLa cells or McCoy cells
- 35 – 37°C
- Cell growth inhibitor
 - DEAE dextran
 - cyclohexamide
 - radiation

not comparable with
in-vivo situation

Chlamydia-host cell interaction

- keratinocytes  33°C
- endothelium
- macrophages
- dendritic cells
- lymphocytes
- cervical cells (ME180)  37°C
- no cell growth inhibitors

Chlamydia strains

- 3 LGV laboratory strains
 - serovars L1, L2 and L3
- 3 LGV recent clinical isolates
 - serovar L2
- Controls:
 - 1 OG strain (serovar E)
 - Chlamydia free McCoy cell culture
 - cell free tissue culture medium

Cell lines

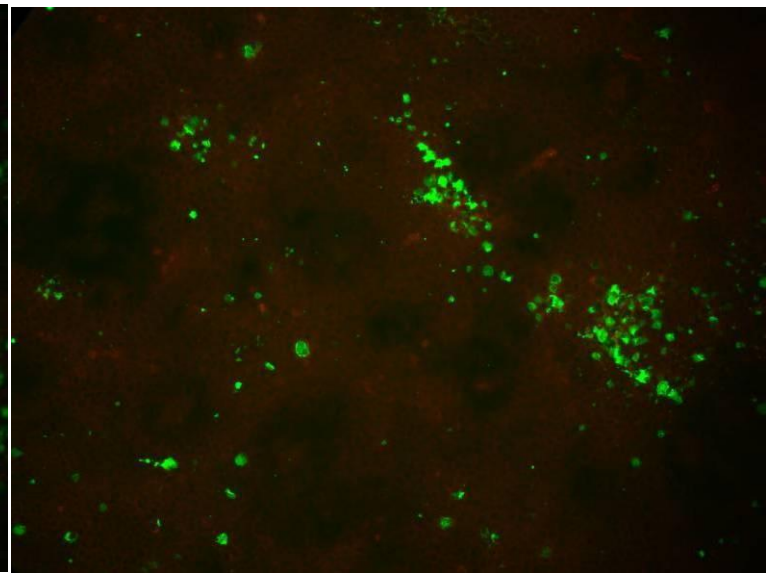
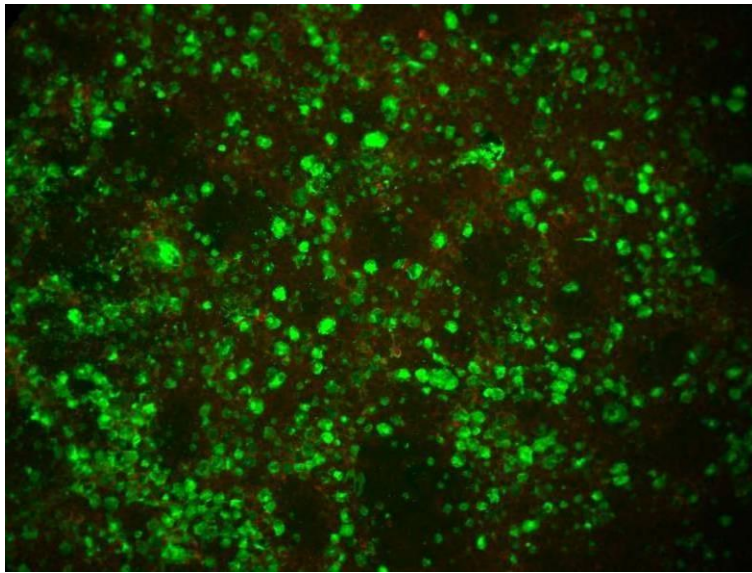
- Keratinocytes
 - HaCaT
- Endothelium
 - HUVEC
- Macrophage
 - M43 cells
- Dendritic cells
 - HB2 cells

Cell lines

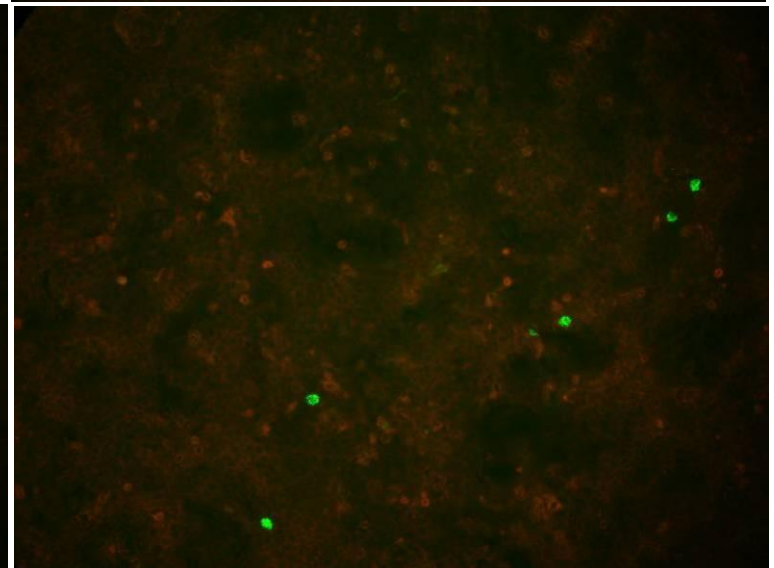
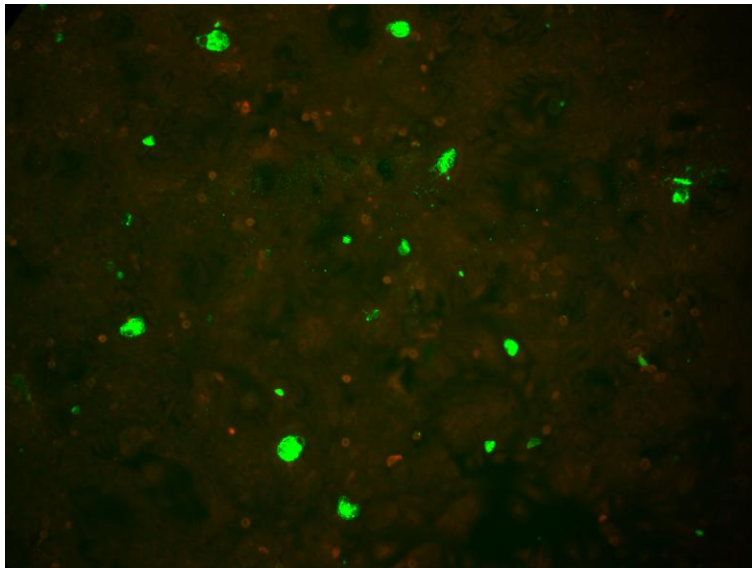
- Keratinocytes
 - HaCaT
- Endothelium
 - HUVEC
- Macrophage
 - M43 cells
- Dendritic cells
 - HB2 cells

HaCaT cells

37°C



33°C

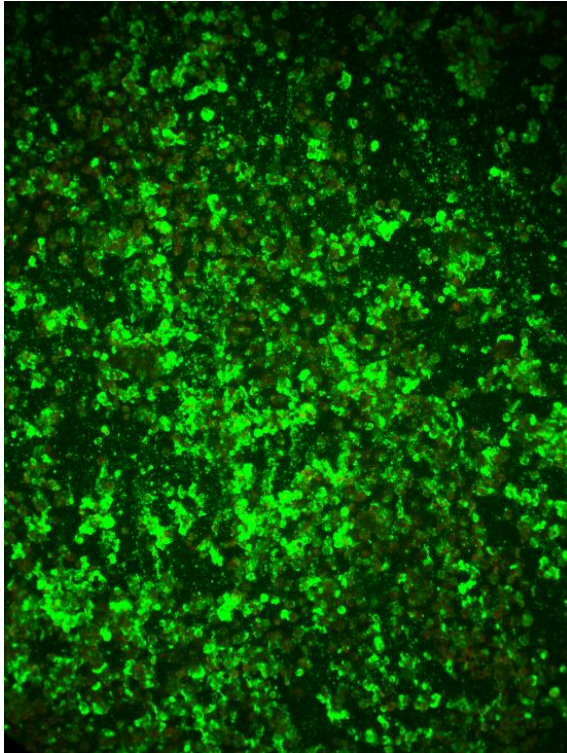


LGV serovar L2
(reference strain)

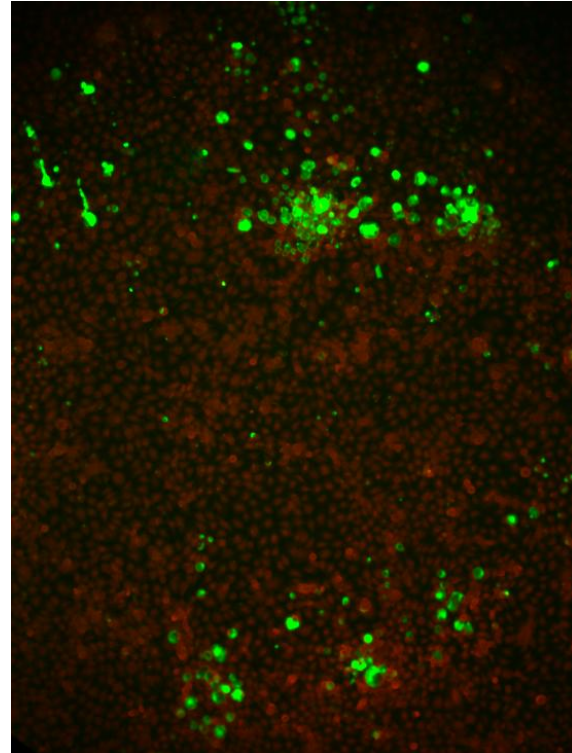
OG serovar E

ME 180 cells

37°C

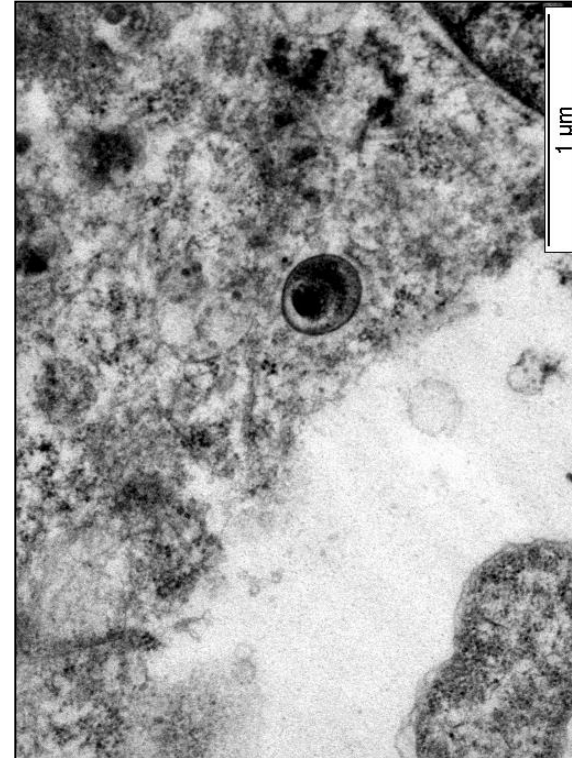
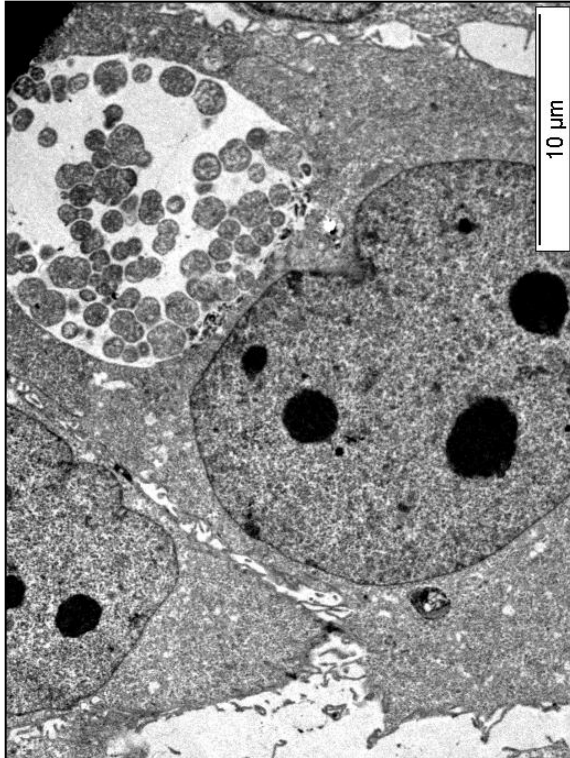


LGV serovar L2
(reference strain)



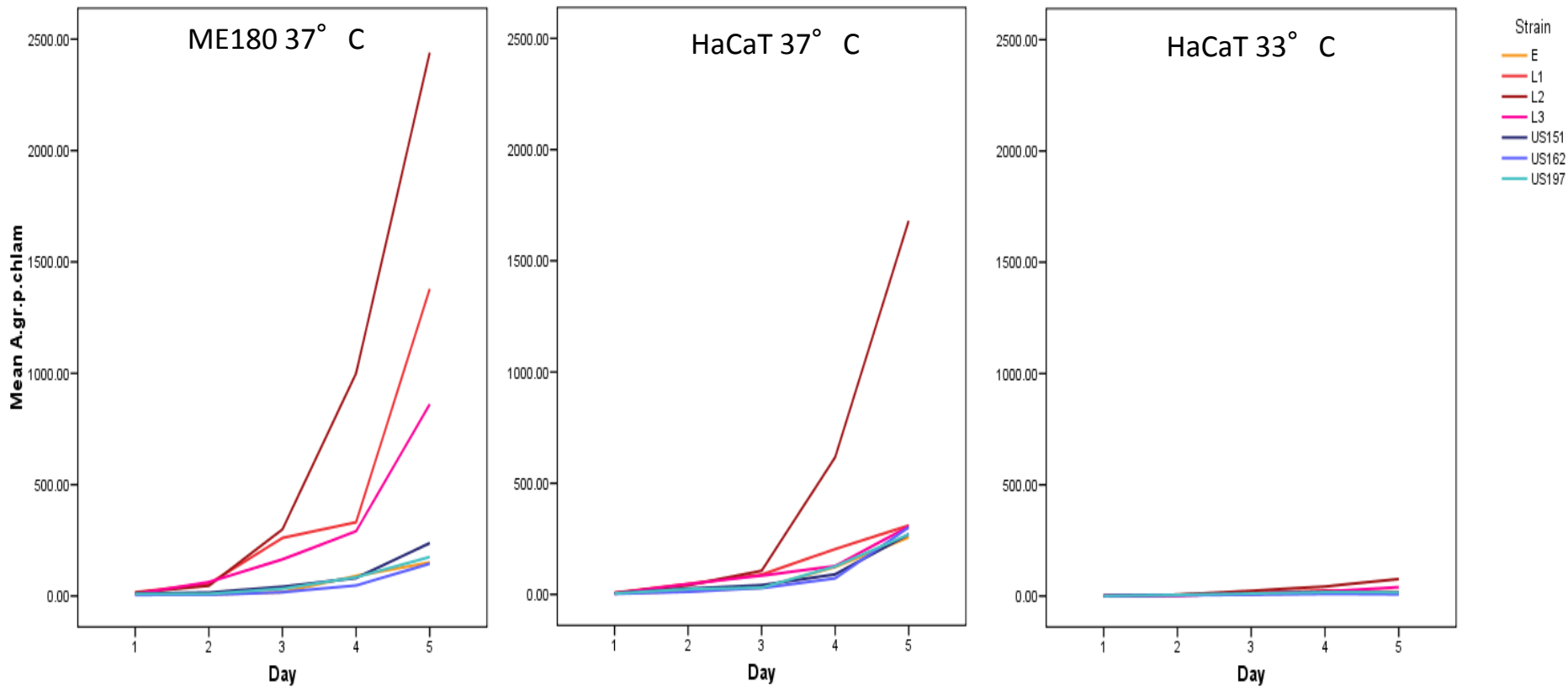
OG serovar E

LGV in HaCaT cells at 33°C



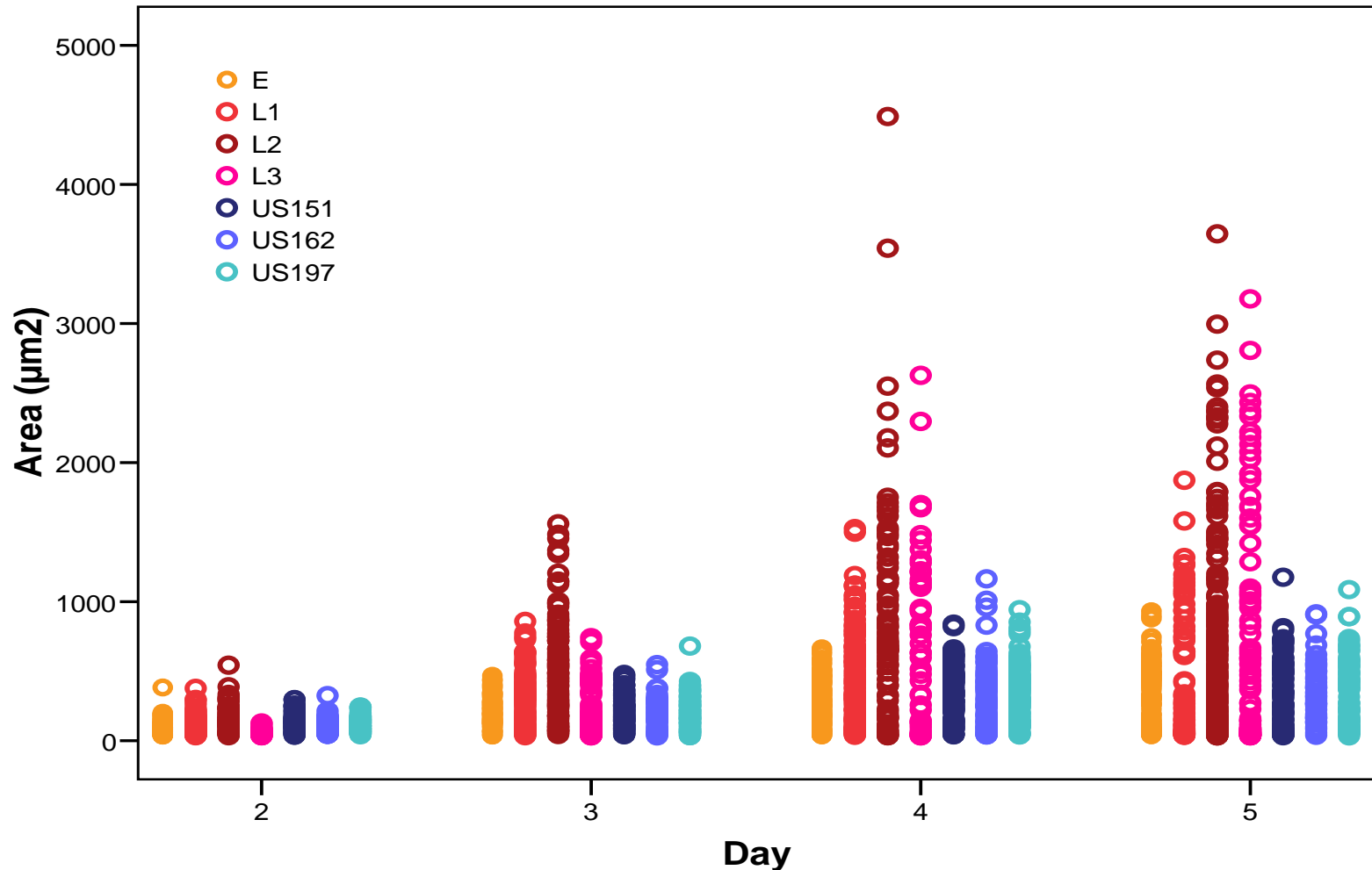
EB in cytoplasm
outside inclusion body

↳ only with LGV at 33°C



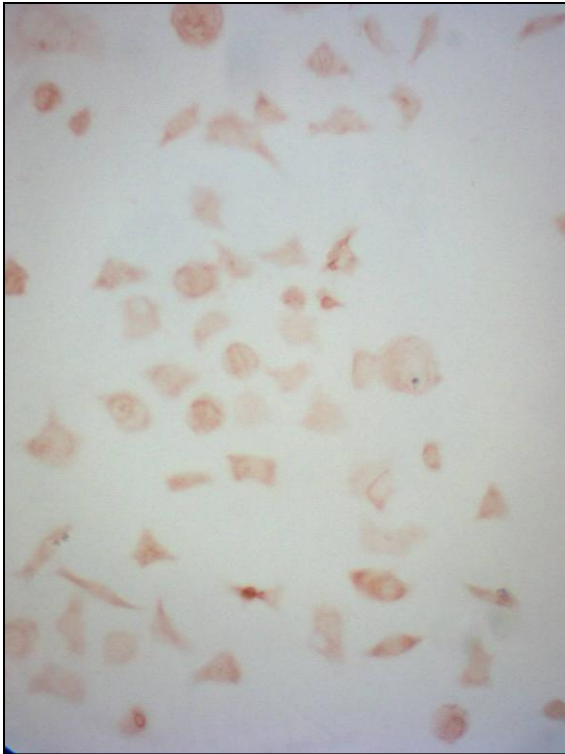
Mean area occupied by Chlamydia per starting organism

Median size of chlamydial inclusions in HaCaT cells at 33° C from 2 to 5 days post infection

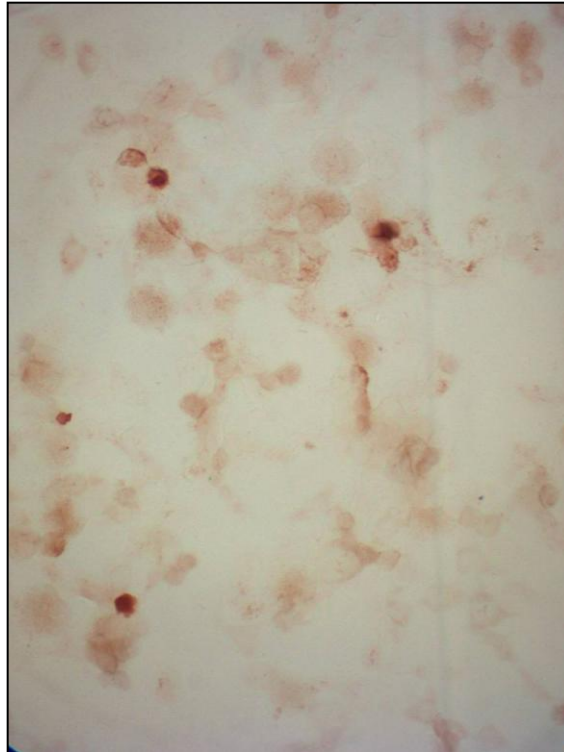


TUNEL assay in ME180

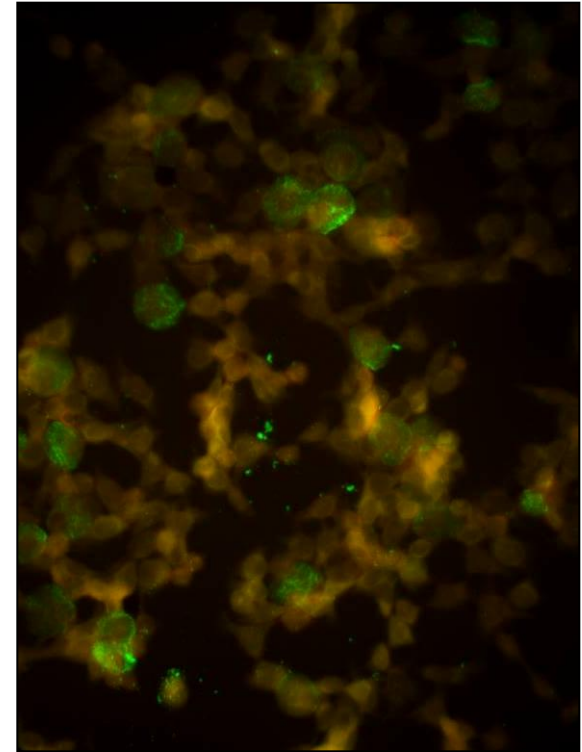
(TdT-mediated dUTP-biotin nick end labeling)



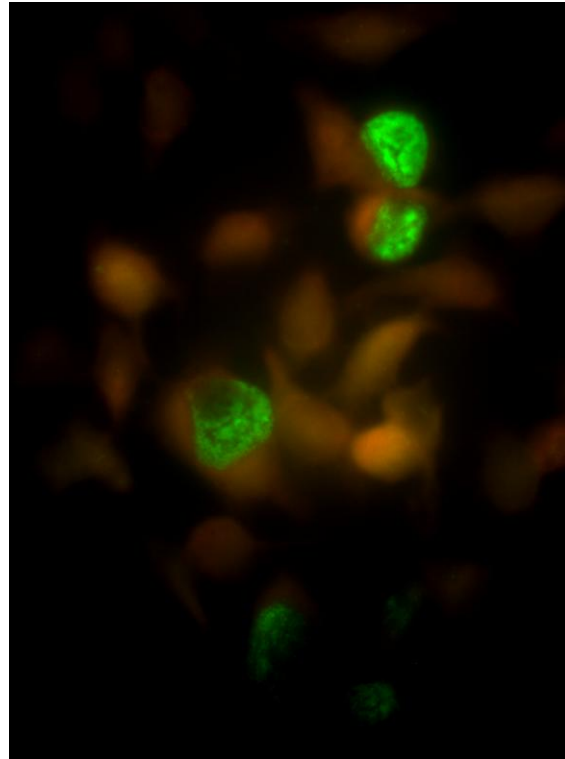
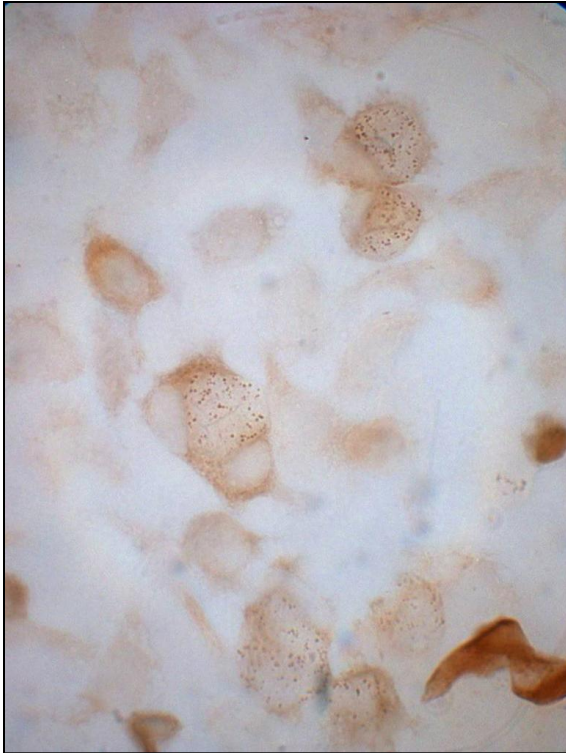
OG serovar E



LGV serovar L2

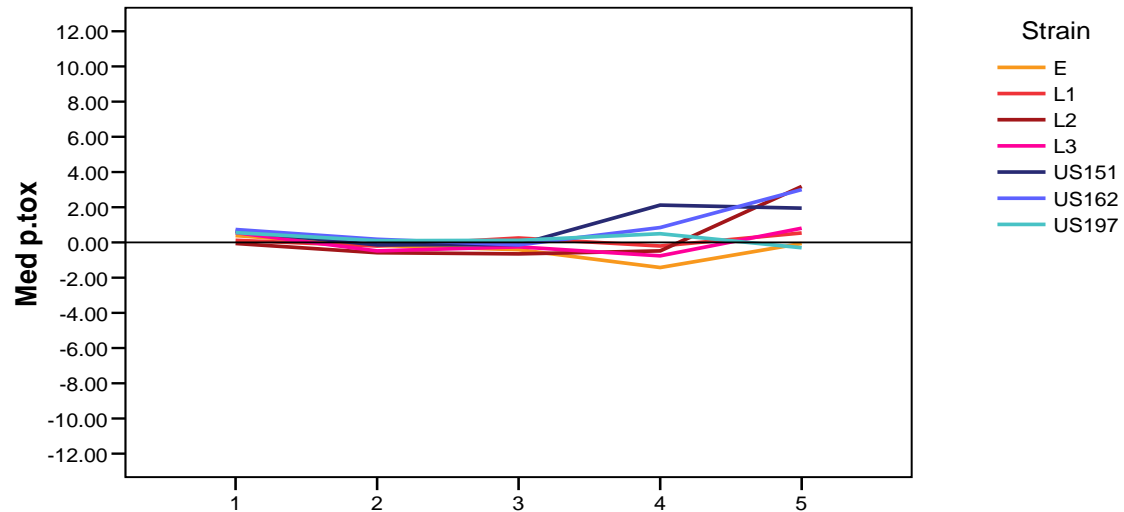


TUNEL assay

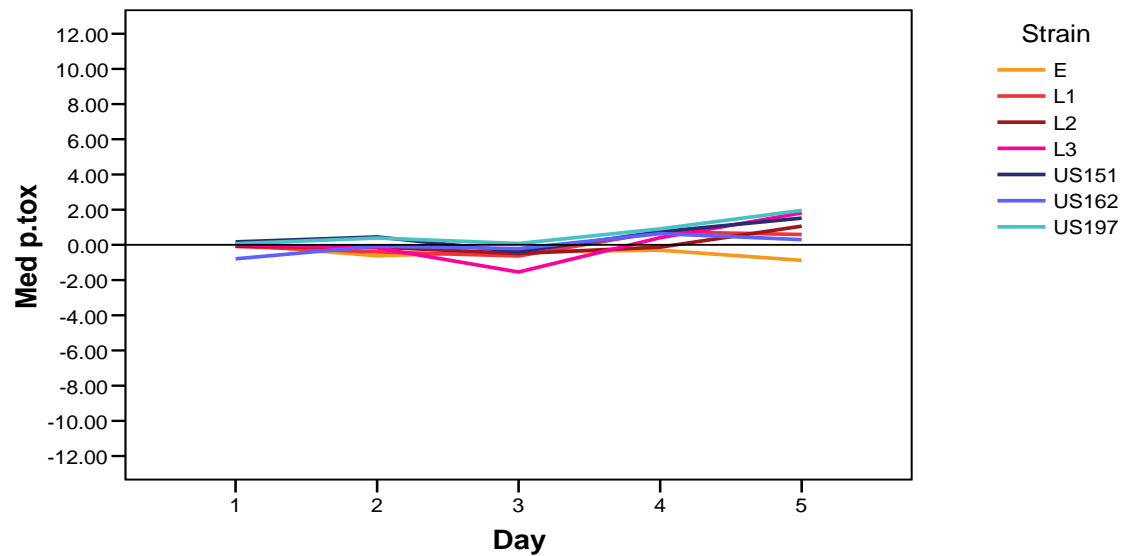


Cytotoxicity in HaCaT cells

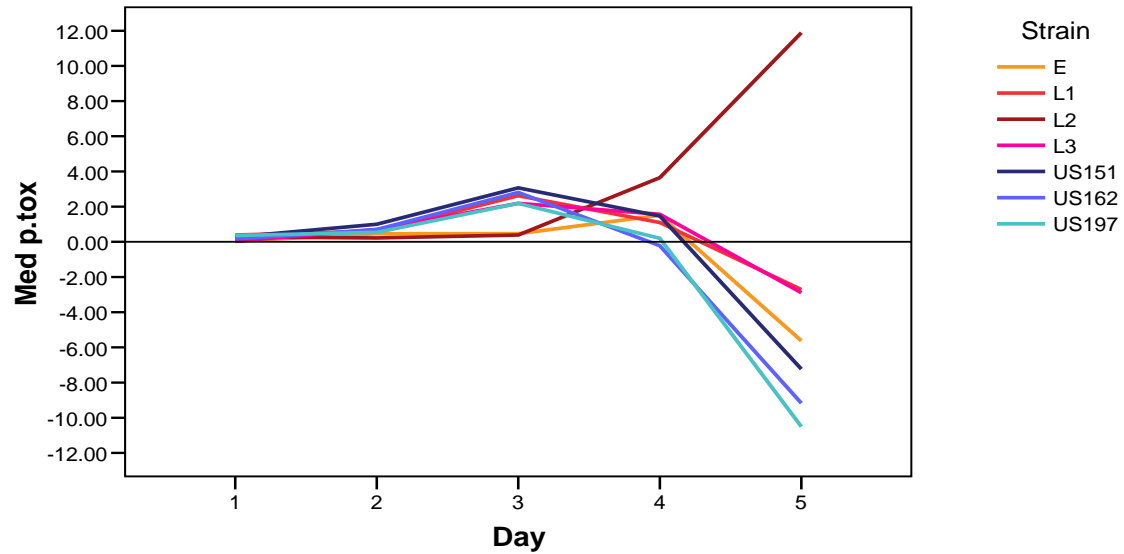
37°C



33°C



Cytotoxicity in ME180 cells



Conclusions

- The LGV reference strain L2 behaves very different from recent clinical isolates of serovar L2



conclusions can only be drawn from work with fresh clinical isolates

- All strains tested grow faster at 37°C as compared to 33°C
- LGV strains induce stronger apoptosis in HaCaT cells as compared to the OG control
- All LGV (but not the OG) strains, kill HaCaT cells at 33°C through necrosis
 - cytotoxin ?
 - LGV specific
- In HaCaT at 33°C, EB bodies of LGV strains escape from the inclusions

Cell lines

- Keratinocytes
 - HaCaT
- Endothelium
 - HUVEC
- Macrophage
 - M43 cells
- Dendritic cells
 - HB2 cells

Extravasation

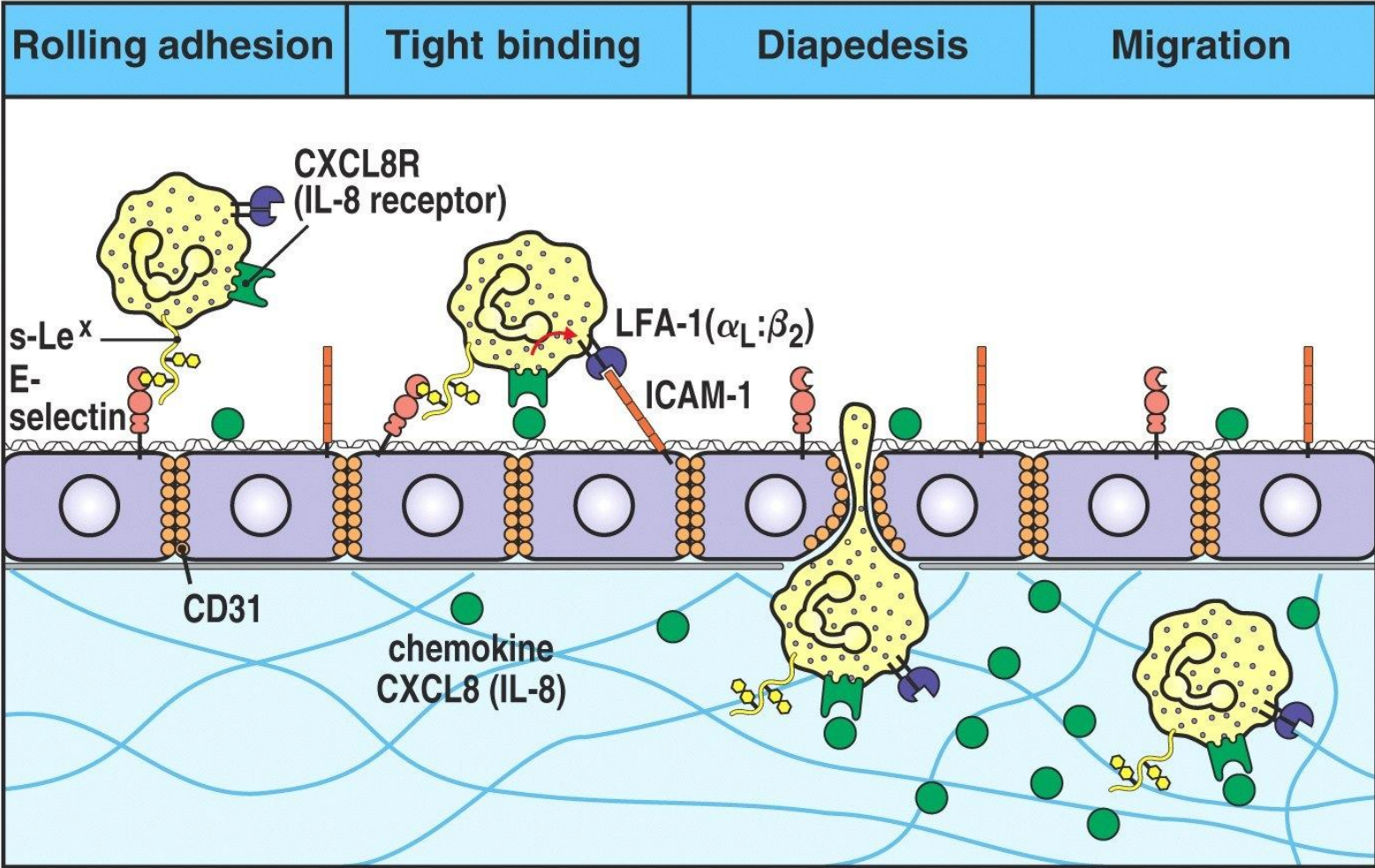
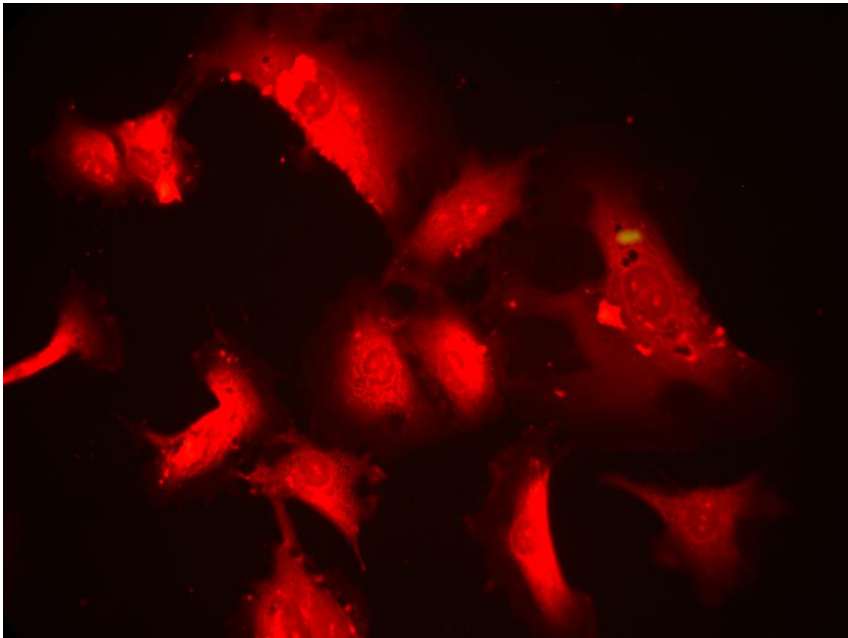
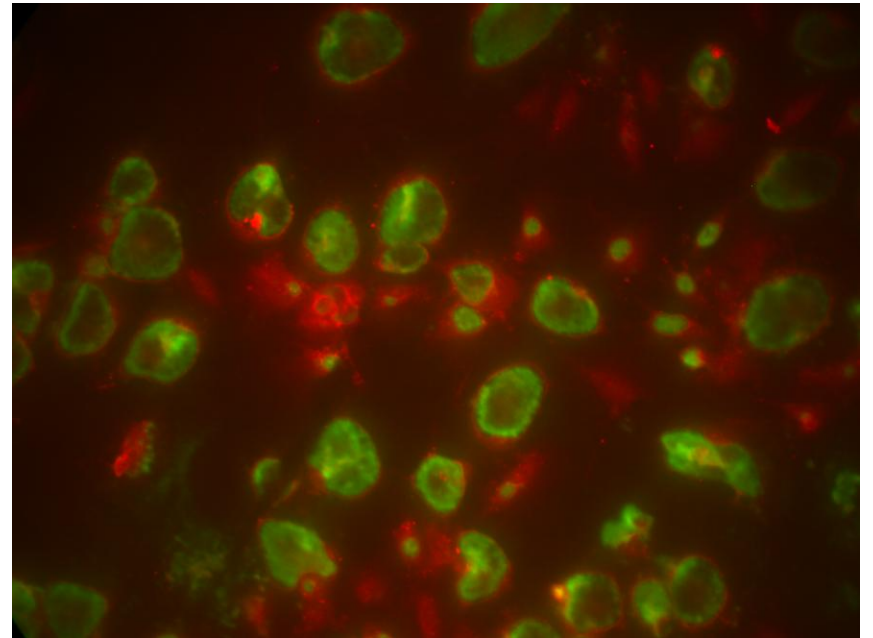


Figure 2-44 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)



Uninfected HUVEC



HUVEC infected with L2

Conclusions

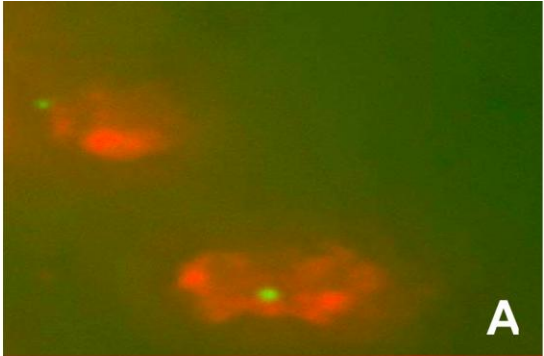
- All *C. trachomatis* strains tested
 - grow in HUVEC cells
 - induce production of IL8 and MIP-1 by HUVEC.
 - induce the expression of ICAM-1 by HUVEC cells
 - induce cell death in HUVEC through apoptosis
 - induce necrotic cell death in HUVEC
- There are quantitative differences between strains
- Limitation: this study used reference strains only

Cell lines

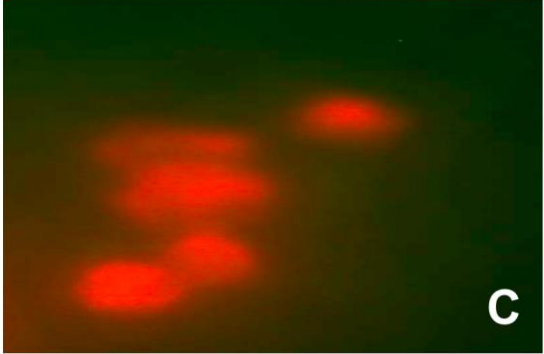
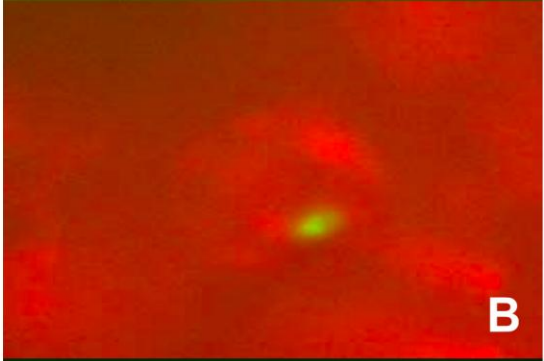
- Keratinocytes
 - HaCaT
- Endothelium
 - HUVEC
- Macrophage
 - M43 cells
- Dendritic cells
 - HB2 cells

Infected macrophages (M43) and dendritic cells (HB2)

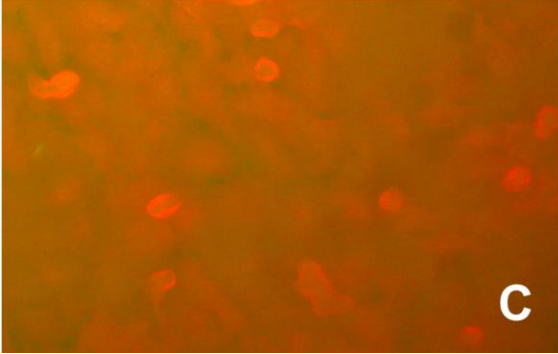
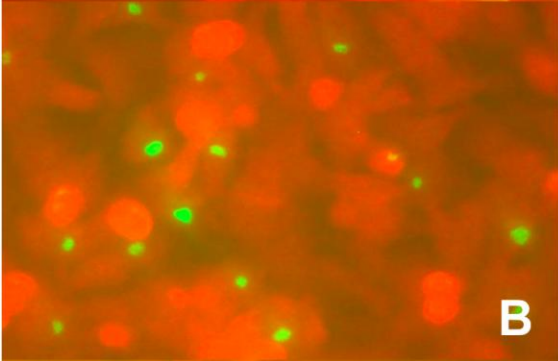
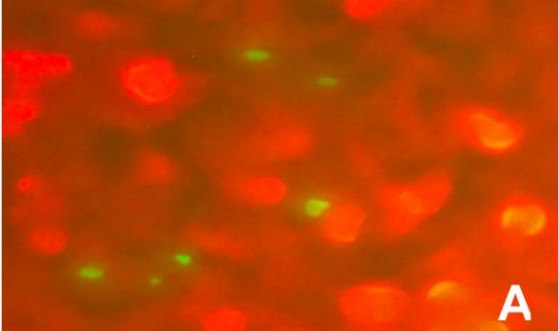
Serovar E



Serovar L2



M43 cells

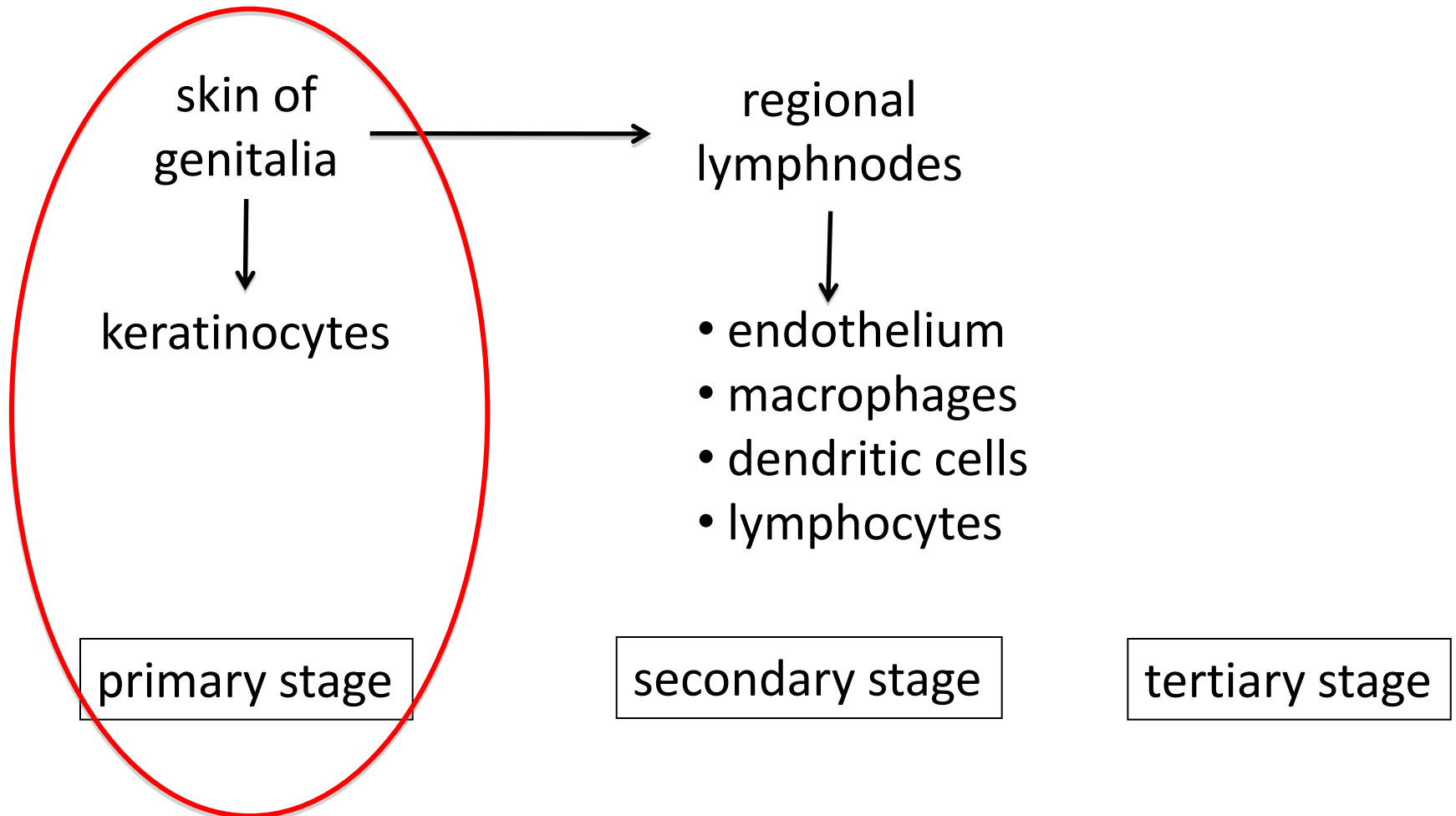


HB2 cells

Conclusions

- Dendritic cells take up more chlamydia than macrophages
- Both LGV serovar L2 and OG serovar E induce pro-inflammatory cytokine production in both macrophages and dendritic cells
- Both L2 and OG serovar E kill macrophages and dendritic cells through necrosis and apoptosis
- Limitation: only 2 reference strains used

Lymphogranuloma venereum



Two new observations

- *C. trachomatis* biovar LGV produces a keratinocyte specific toxin
 - characterisation of toxin in progress
- EB of LGV strains escapes from the inclusion bodies in keratinocytes when grown at 33°C
 - allows for early escape to the lymphnodes ?

Acknowledgments

- Dr. Bronwyn Joubert
- Ikanyeng Dolly Seipone
- Yoshen Moodley
- Adele Cheddie