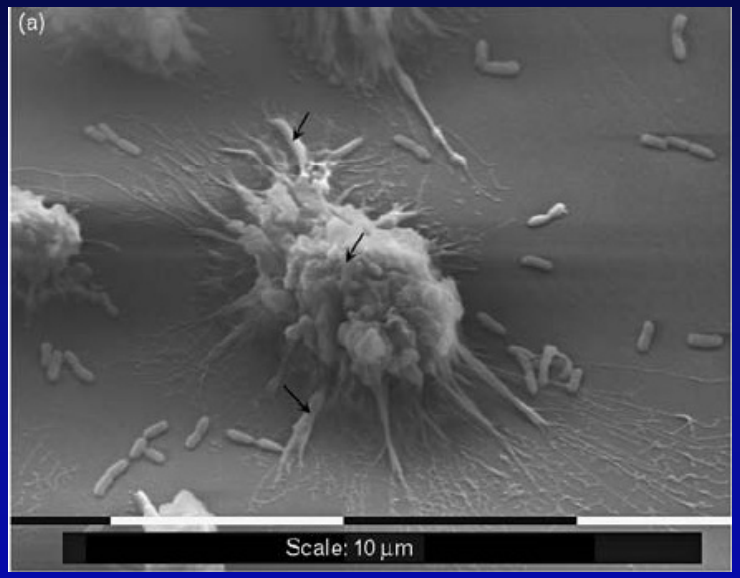
Role of capsule, lipopolysaccharide and flagella in adherence, invasion and intracellular survival of *Burkholderia pseudomallei* inside human lung epithelial cell line

Miss Chanthiwa Wikraiphat

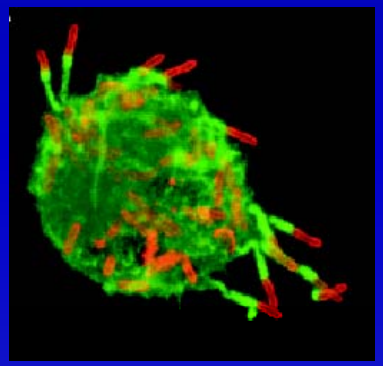
Faculty of Science, Mahidol University

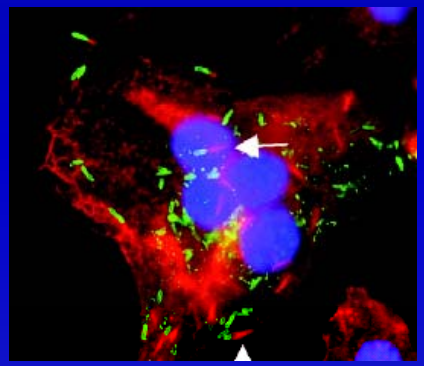
Faculty of Tropical Medicine, Mahidol University

B. pseudomallei



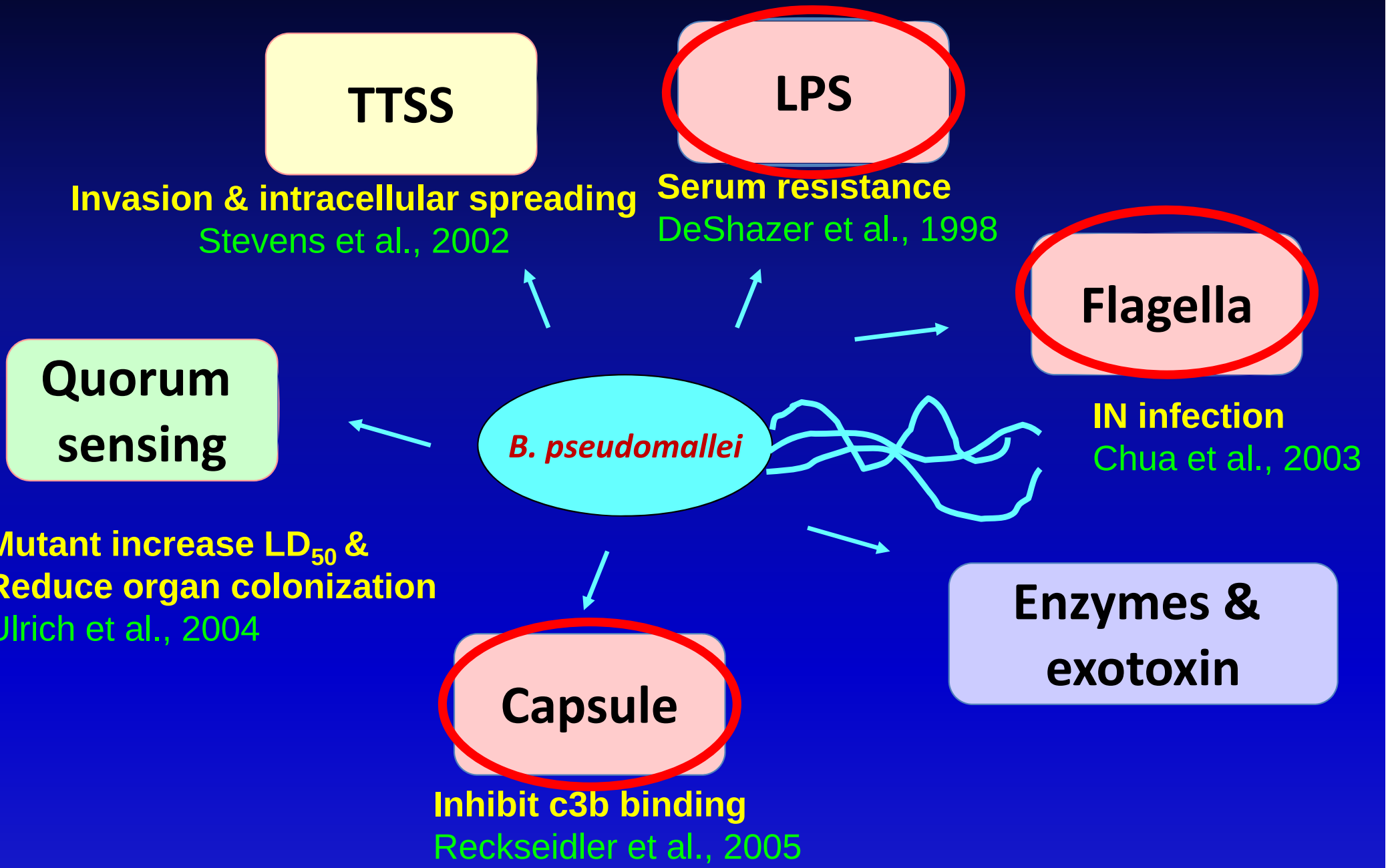
Wiersinga & van der Poll, 2009





- facultative intracellular pathogen
- cause of melioidosis
- endemic in Southeast Asia and northern Australia
- Group B biological agent
- survive in phagocytic and non-phagocytic cells
- resistance to complement-killing, antimicrobial peptides
- induce membrane protrusion
- lead to multinucleated giant cells (MNGCs) formation

Virulence determinants

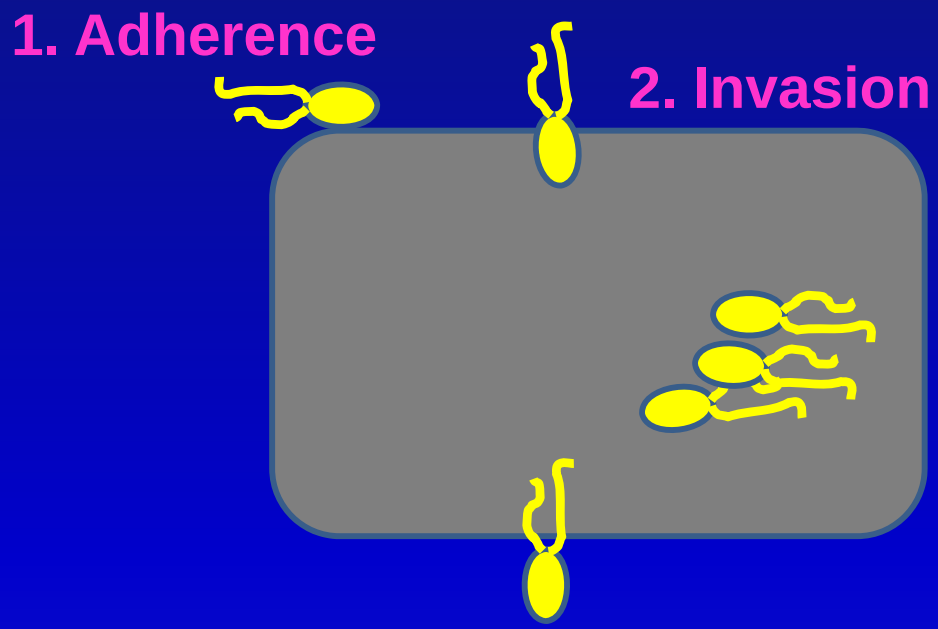


Objectives

To investigate the role of capsule, LPS and flagella of *B.pseudomallei* as virulence factors in adherence, invasion and intracellular survival in human epithelial cell.

Study design

Infected epithelial cell line (A549) with *B. pseudomallei*



Materials and Methods

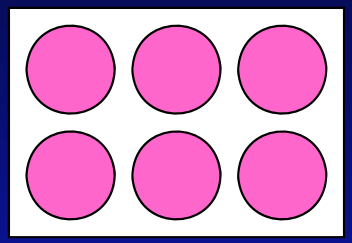
B. pseudomallei strains

(provided by Prof. DE Woods)

Strains	Characteristics
wild type	clinical isolate, Tc^S
CPS mutant	*wcb*::pSR1015, Tc^R
LPS mutant	*wbil*::Tn-OT182, Tc^R
flagellin mutant	*fliC*:: Tn-OT182, Tc^R

1. Adherence assay

A549 90% confluency

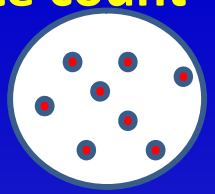


B. pseudomallei WT and mutants MOI 10:1

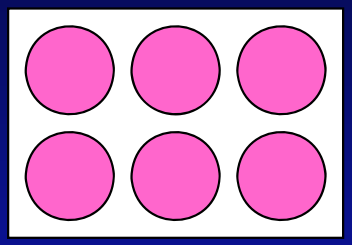
37˚C, 1 h

Lysed cell with 0.1% Triton X-100

Viable plate count



A549 90% confluency



B. pseudomallei WT and mutants MOI 10:1

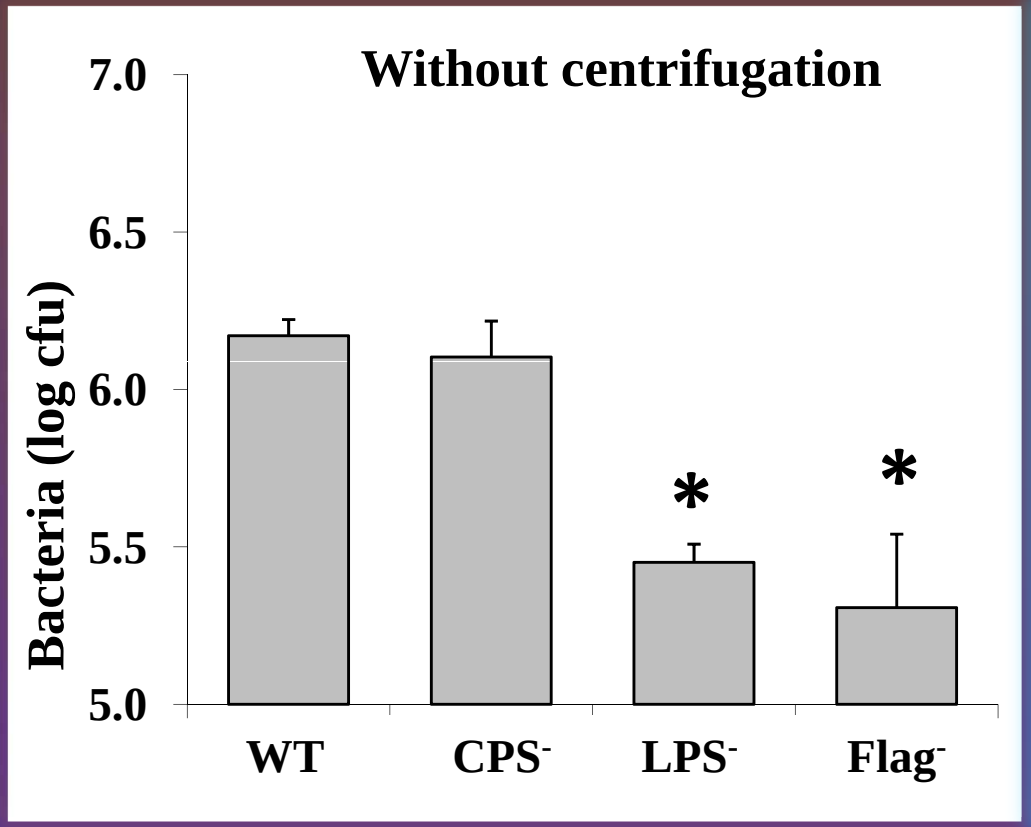
Centrifuge at 165 xg, 5 min

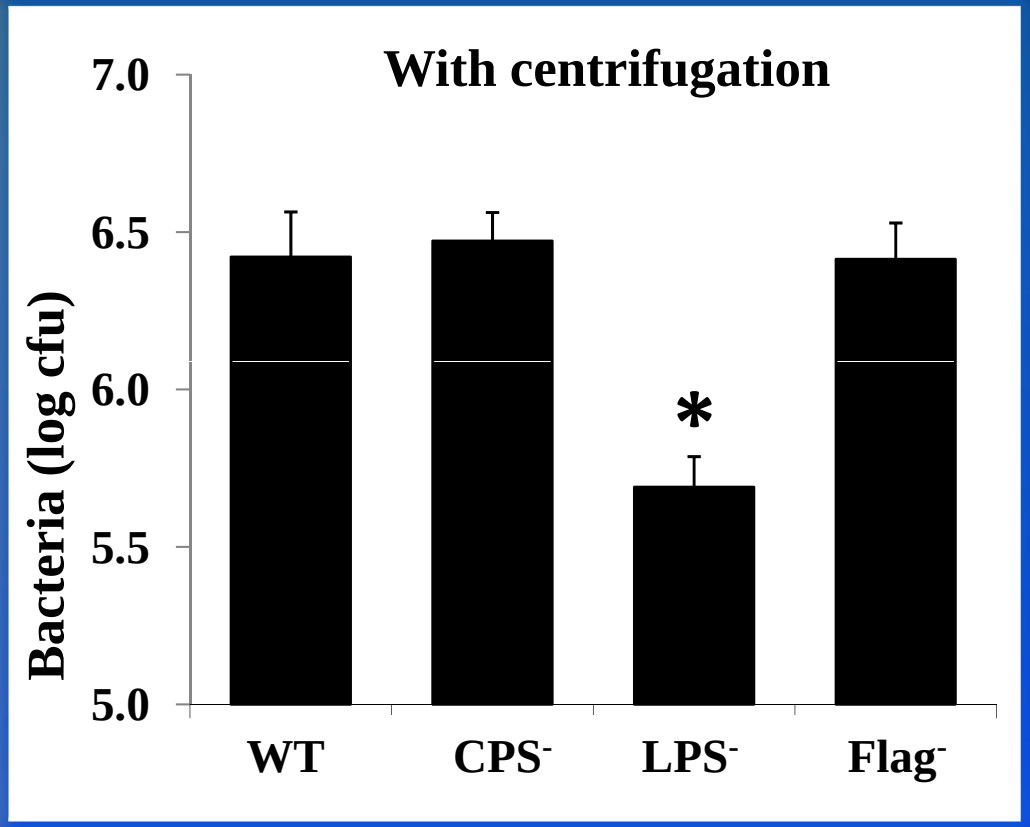
37˚C, 1 h

Lysed cell with 0.1% Triton X-100

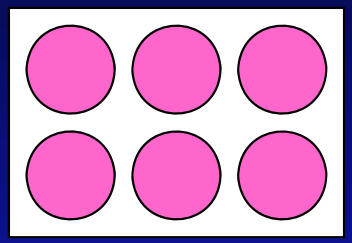
Inglis et al.,Infect immun 2003

Adherence to epithelial cell





2. Invasion assay



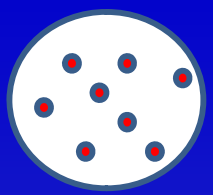
B. pseudomallei WT and mutants MOI 10:1

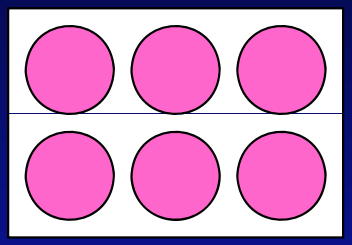
37˚C, 2 h

Kanamycin 250 μg/ml

37˚C, 1 h

Lysed cell with 0.1% Triton X-100



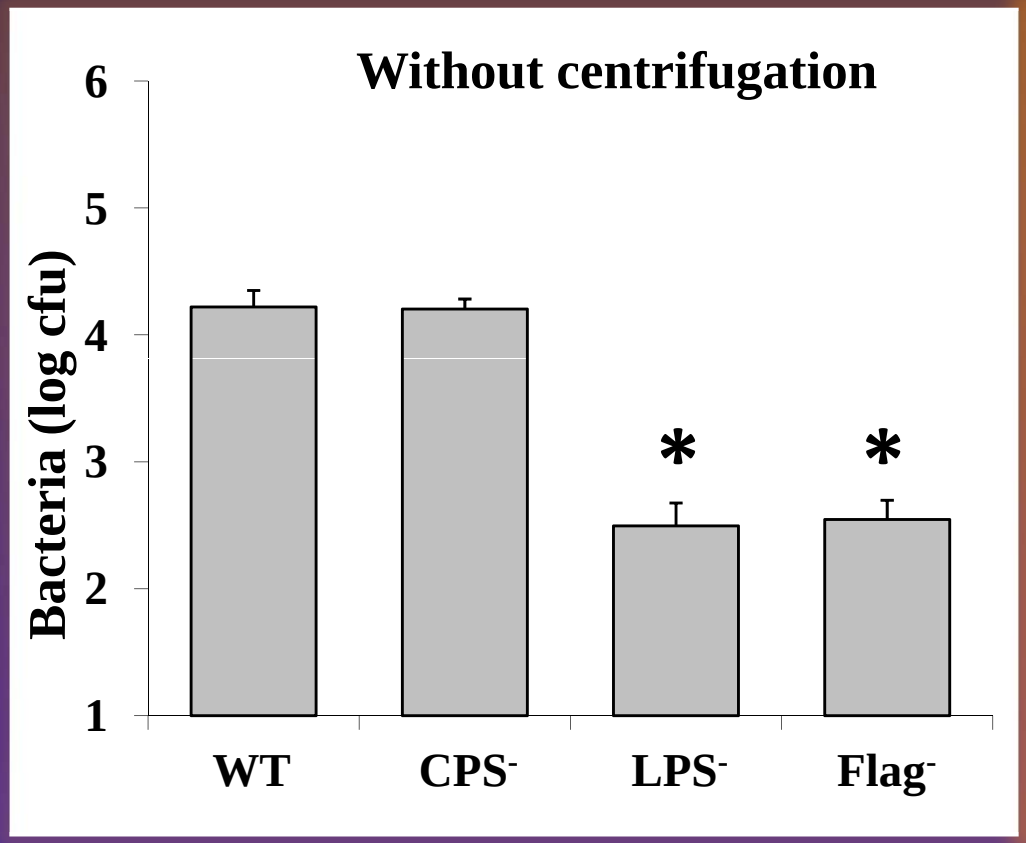


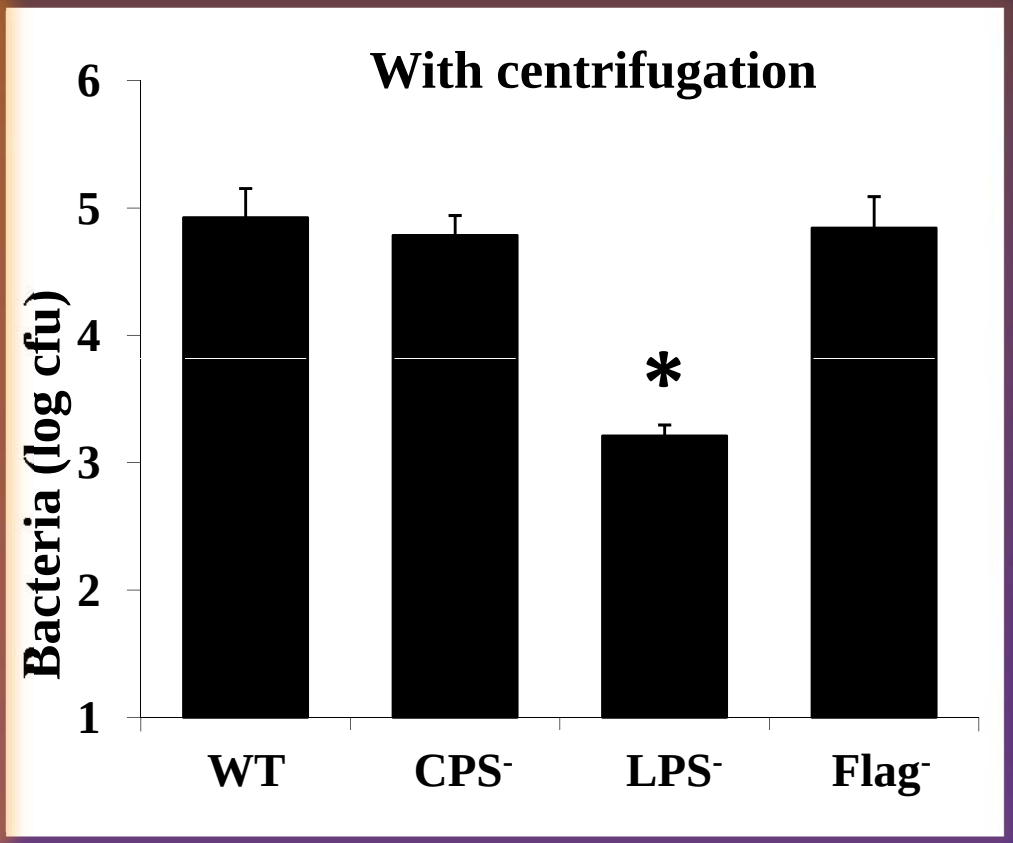
B. pseudomallei WT and mutants MOI 10:1

Centrifuge at 165 xg, 5 min

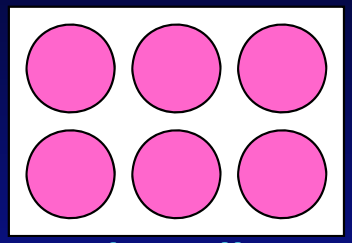
Utaisinhacharoen et al., Microb Pathog 2005

Invasion into epithelial cells





3. Intracellular bacteria



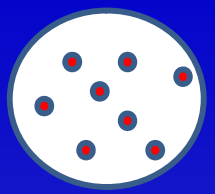
B. pseudomallei WT and mutants MOI 10:1

37˚C, 2 h

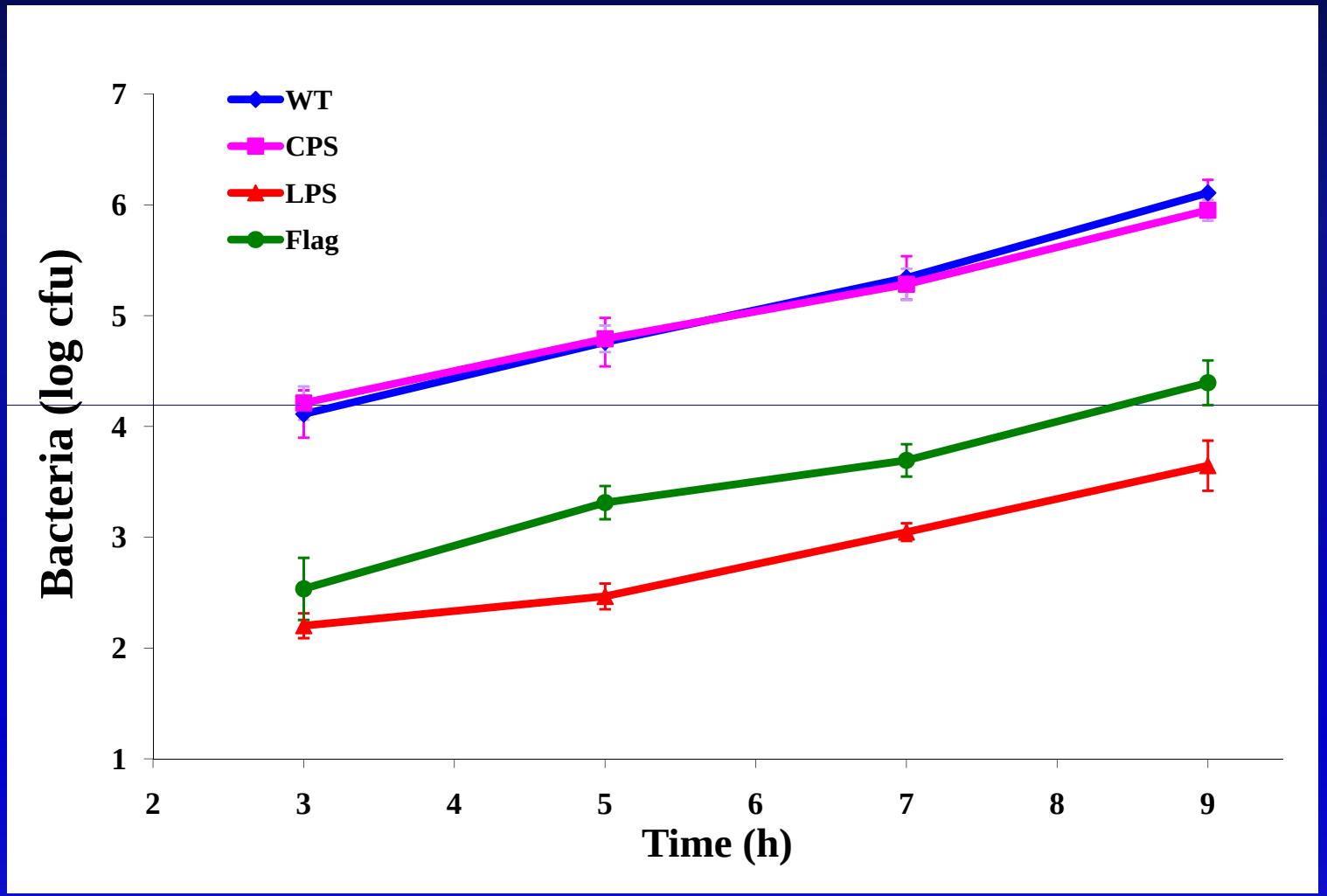
Kanamycin 250 μg/ml

37˚C, 2 h

Kanamycin 20 μg/ml



Intracellular survival and multiplication in epithelial cell



Doubling time of intracellular bacteria

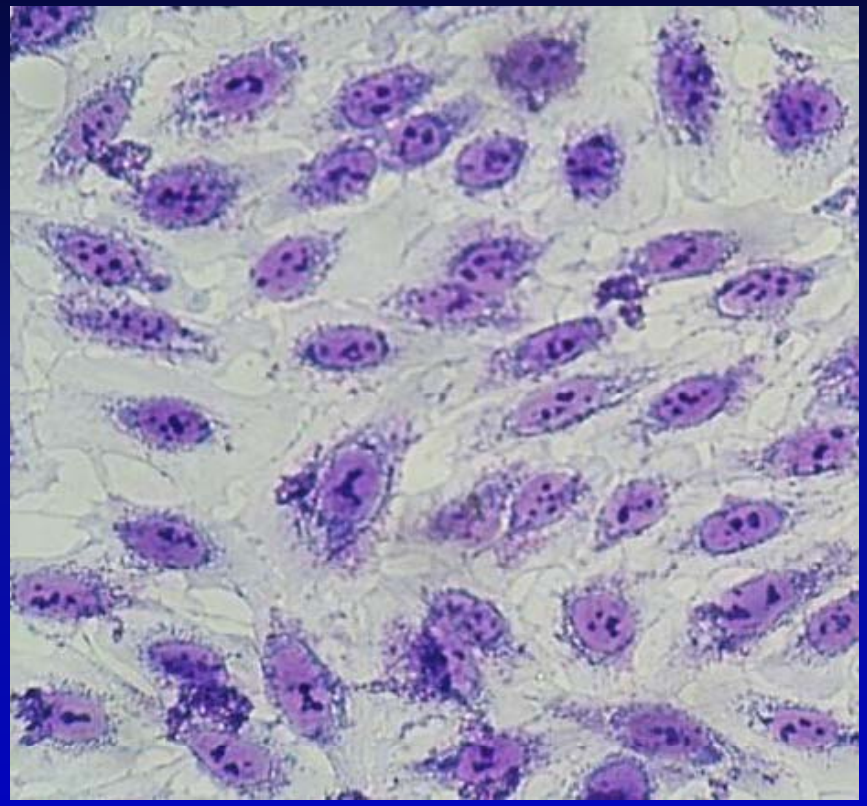
WT: 57 min

CPS: 62 min

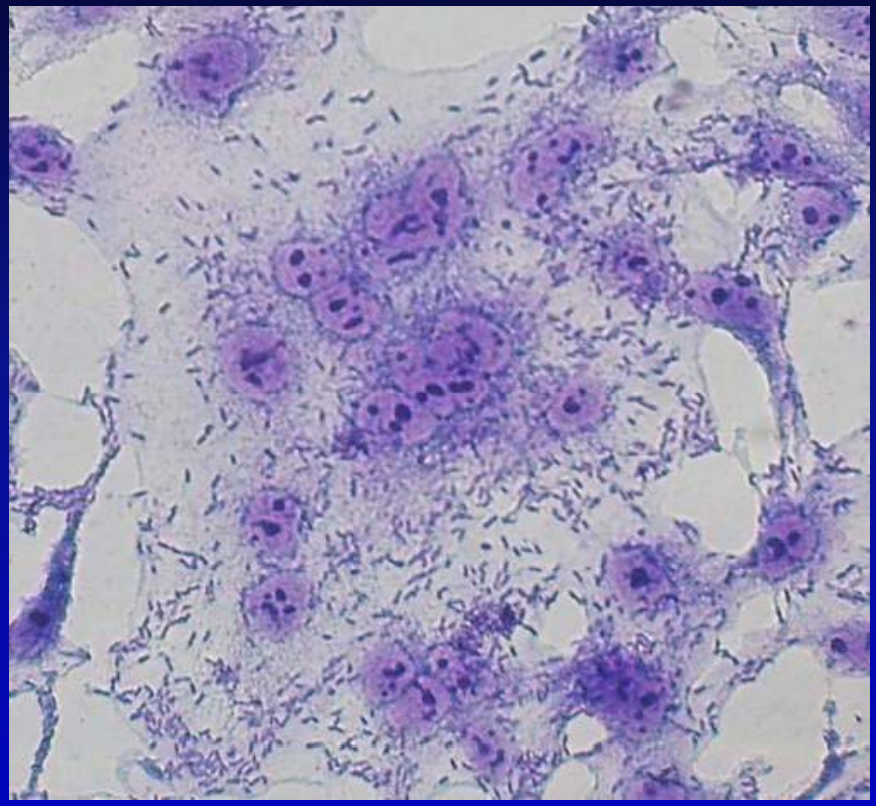
LPS: 57 min

Flag: 62 min

Multinucleated giant cell (MNGC) formation



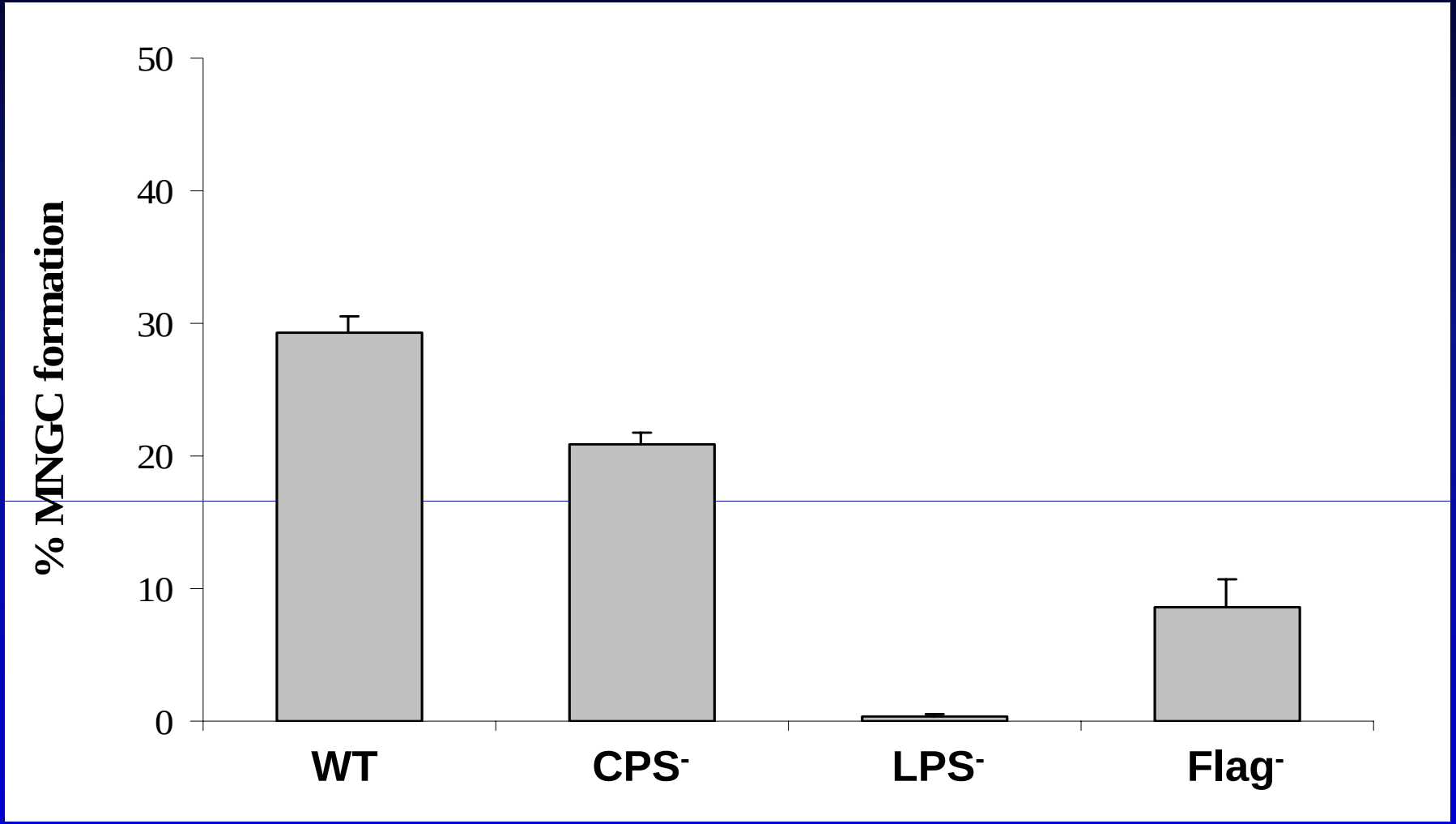
Normal cells



MNGCs

$$\% \text{ MNGC} = \frac{\text{no. of nuclei within MNGC} \times 100}{\text{Total no. of nuclei counted}}$$

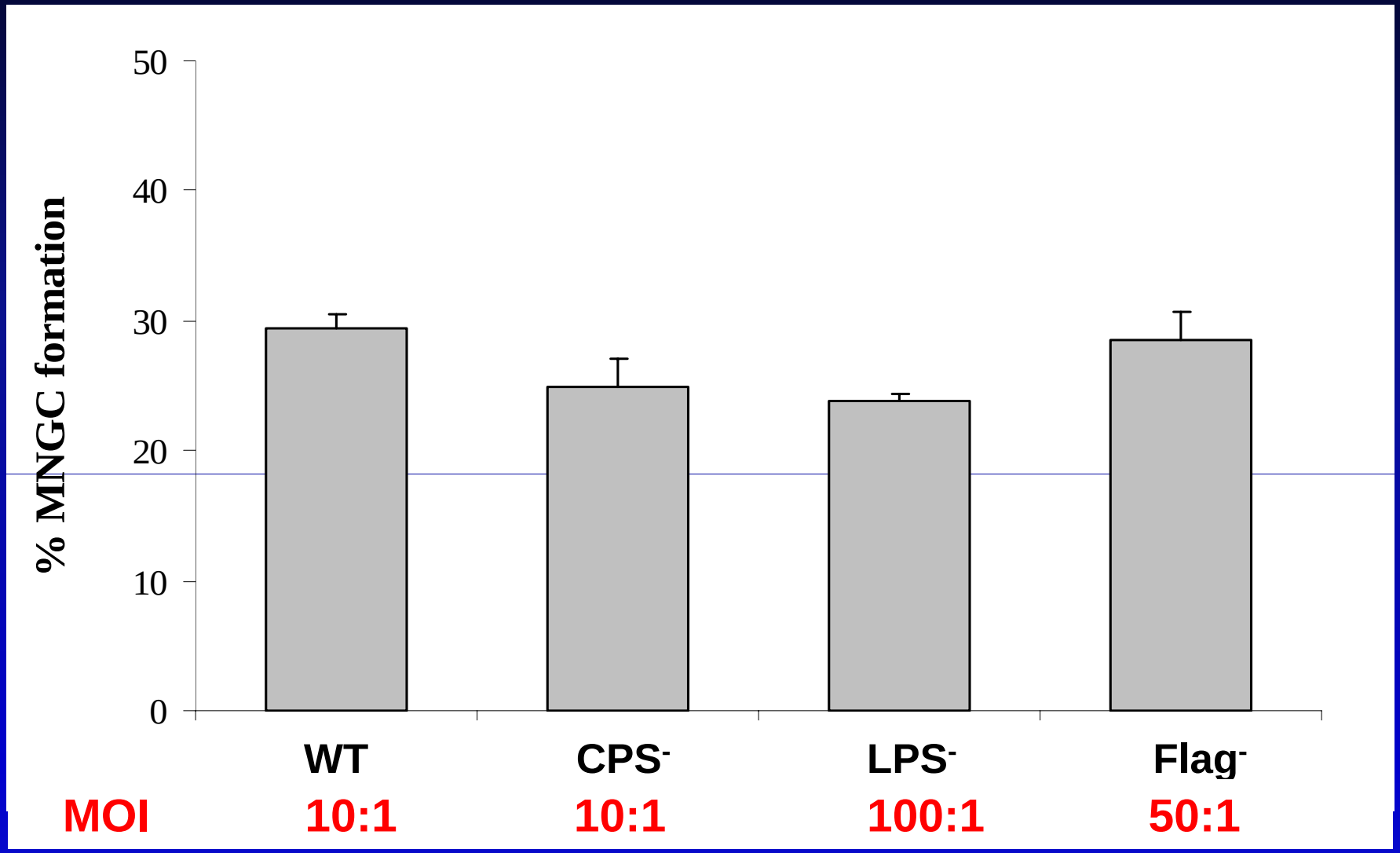
MNGCs formation



Low MNGC formation by LPS and flagellin mutants: defective of mutants / number of intracellular bacteria

MNGC formation

(increase MOI of LPS$^-$ and Flag$^-$)



MNGC formation related to no. of intracellular bacteria.

Discussion

- **O-PS of LPS is involved in adherence and invasion of *B. pseudomallei* to epithelial cells.**

  - *Salmonella* & *Shigella* (West et al., 2005)

  - may act as bacterial ligand: adhesion

  - affect TTSS function: invasion

  - EM: polysaccharide layer facilitate attachment of *B. pseudomallei* to pharyngeal epithelial cells (Ahmed et al., 1999)

Discussion (Conc.)

- **Flagella facilitate motility of bacteria to increase contact with host cells.**

- penetrate mucous in IN infection of animal model (Chua et al., 2003)

- **Capsule, LPS or flagella are not required for intracellular survival and MNGCs formation of *B. pseudomallei* in epithelial cells.**

Acknowledgements

- Department of Microbiology, Faculty of Science, MU
  - Prof. Stitaya Sirisinha
  - Assoc. Prof. Pongsak Utaisincharoen
  - Member of K603-605 & Staff at Microbiology
- Department of Microbiology and Immunology, Faculty of Tropical Medicice, MU
  - Dr. Narisara Chantratita
- The Institutional Strengthening Program, Faculty of Science, MU
- RA Scholarship, Faculty of Graduate Studies, MU
- Mahidol-Oxford Tropical Medicine Research Unit