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Quantitation of fluorescent chlamydial gene reporter expression by flow cytometry enables investigation of responses to immunological and environmental stress in real time

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Abstract

Background: *Chlamydia trachomatis* (Ct) and *C. muridarum* (Cm) are obligate intracellular bacteria and investigating gene expression by these pathogens can be challenging. We have implemented flow cytometric assays to quantify expression of fluorescent reporters in real time.

Methods: Epithelial cells were infected with Ct or Cm expressing *groEL::*mCherry, *omcA::gfp* or *glgA::gfp* and subjected to IFNγ-mediated tryptophan restriction or glucose limitation. Infection was monitored by microscopy over the course of 40 hours. Infection was quantified (1) by counting mCherry and/or GFP-expressing inclusions in 20X fields acquired by microscopy or (2) by determining frequency and fluorescence intensity of GFP and mCherry using flow cytometry.

Results: IFNγ pretreatment of infected cells resulted in decreased *omcA* transcription by mCherry+ Ct inclusions, indicating that *omcA::gfp* is a useful tool for studying persistence. Results acquired by flow cytometry reflected those obtained by direct counting of GFP+ and mCherry+ inclusions in imaged cells. The *glgA::gfp* reporter appeared weaker than *omcA::gfp* although both reporters were down-regulated in response to glucose limitation. Assaying *glgA::gfp* in *uhpC* mutants, we observed striking upregulation of expression compared to the reporter in the wild type parent.This 'dim' to 'bright' phenotype was easily quantified by flow cytometry but difficult to measure by microscopy.

Conclusion: Flow cytometry quickly and accurately quantified transcription of fluorescent reporters fused to Ct or Cm genes. This is a useful technique that facilitates real time investigation of chlamydial persistence in mutant strains and may be broadly applied to kinetic profiling of chlamydial gene expression.