Characterizing Chlamydial Cytotoxins as Inflammatory Modulators: C. Muridarum and C. Trachomatis Putative Glycosyltransferase Activity and NF-kappa B Regulation

Shivnani, Vijay (School: Plano West Senior High School)

Chlamydia trachomatis (Ctr) causes over 130 million urogenital infections annually. Despite high genetic similarity, Chlamydia muridarum (Cm) exhibits different host tropism. Interspecies divergence may be driven by variation in the cytotoxin (tox) locus (Cm TC0437-0439; Ctr CT166), exhibiting sequence similarity to glycosyltransferases (GTs). Although initially hypothesized to induce immediate cytotoxicity, recent evidence suggests the tox locus instead exacerbates oviduct immunopathology. This study investigated cytotoxin-mediated inflammation by (1) assessing NF-kappa B activation in Cm tox mutant infections, (2) modeling glycosyltransferase activity computationally, and (3) purifying CT166 for antibody development. HeLa cells infected with a wild-type Cm Nigg, a cytotoxin-deletion mutant, and a complemented strain were assessed for NF-kappa B proteins by Western blotting. At 12, 24, and 48 hours post-infection, I-kappa B-alpha phosphorylation and p65 abundance did not vary. The tox locus likely does not directly modulate the proposed oviduct-driven immune pathway. A pivot from host responses to the Chlamydial mechanisms for prospective therapy efficacy was examined with GT activity: predicted TC0438 and CT166 structures were docked with UDP-sugar donors and acceptors. TC0438 complexes displayed higher RMSD and HADDOCK scores than CT166, suggesting greater Ctr dependence on Rho-GTPase inactivation. For GT validation, a cytotoxin antibody is desirable; CT166 production and purification were optimized in an ectopic E. coli production model for decreased protein misfolding. Chlamydial cytotoxins likely drive inflammation through GT activity rather than I-kappa B-alpha signaling. GT inhibition strategies should be examined for novel chlamydia-induced infertility therapy.

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