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The gonococcal pilin-linked glycan modulates the host innate immune response in a primary cervical cell-macrophage co-culture model of infection

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Background

Type IV pili of Neisseria gonorrhoeae (Ng) contribute to host immune avoidance and the ability of these bacteria to initiate disease. Ng pili are post-translationally modified with an O-linked, galactose(α1-3)-2,4-diacetimido-2,4,6-trideoxyhexose (Gal-diNAcBac) disaccharide. Pilin glycosylation involves multiple pilin glycosylation (i.e., pgl) gene products. PglD contributes to the biosynthesis of diNAcBac, and PglA adds a hexose to this diNAcBac basal sugar. Whereas pglA is subject to phase variation, pglD expression is not.

Aim/Methods

As the biological consequences of pilin-linked glycan (PLG) phase variation are ill-defined, we initiated studies to determine if glycan variation affects complement receptor 3 (CR3)-mediated cervical infection. Primary human cervical epithelial (Pex) cells were infected with wildtype (WT) Ng 1291 or its isogenic mutants, 1291pglA or 1291pglD, and Ng survival, intracellular trafficking, and the host cytokine response to infection were examined.

Results

Modified gentamicin survival assays indicated that, at 3h post-gentamicin treatment, 1291WT (disaccharide PLG) exhibited a dramatic survival advantage over 1291pglA (monosaccharide PLG) and 1291pglD (no PLG). Confocal microscopy revealed that all strains co-localized with intracellular markers associated with an endocytic recycling pathway, consistent with CR3 trafficking, albeit differences were observed. Cytokine array analysis of 24h Pex cell infection supernatants suggested that the absence of the PLG, a scenario that does not occur in vivo, triggered a more robust pro-inflammatory response versus WT Ng. To confirm these data, Pex cells in apical transwell chambers were co-cultured with primary macrophages (present in the basal

chamber). Pex cells were infected with Ng for 24 or 48h. Supernatants were then analyzed for secreted cytokines, and surface and internal markers of macrophage polarization were characterized. All infections triggered a pro-inflammatory response, which was more robust for infections performed using the pgl mutants, suggesting that the Gal-diNAcBac PLG tempers the host response to infection. Macrophages exhibiting both M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotypes were observed. However, the proportion of M2 cells decreased over time, with M1 cells becoming 2-5-fold more predominant by 48h post-infection.

Conclusions

These results are in contrast to those reported previously for direct macrophage infections and suggest that co-culture systems can potentially provide a better understanding of the systemic response to infection.