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Development of tools to characterise antibody and complement-mediated killing of Neisseria gonorrhoeae

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Background

Immune correlates of protection have been invaluable for the design and evaluation of vaccines for many diseases. Serum bactericidal activity (SBA) is an accepted correlate of protection for meningococcal capsular polysaccharide and protein-based vaccines. However, the complex interaction of Neisseria gonorrhoeae with the human host and the lack of protective immunity against reinfection has hampered the development of vaccines to prevent gonorrhoea. Despite this, modest reductions in gonococcal infections have been seen following the use of protein-based meningococcal vaccines. Many tools will be required to determine immunological mechanisms of protection against gonorrhoea with a single correlate of protection unlikely.

Robust and reproducible SBA and opsonophagocytosis assays (OPA) will be an essential component of this analysis.

Aim/Methods

We have developed assays to characterise killing of N. gonorrhoeae by antibody and complement-mediated phagocytosis and bactericidal activity. Mouse anti-outer membrane vesicle or anti-Bexsero serum was incubated with N. gonorrhoeae and 25% IgG and IgM-depleted human plasma (Alexander et al. 2022) for 45 min to determine bactericidal activity. CFU determination was made by plating on chocolate + polyvitex agar using the streak method and incubation for 24h at 37oC. OPA was determined by adding differentiated HL60 cells to the above and, to eliminate membrane attack complex killing, anti-C7 mAb (1.0mg/ml, Quidel) was added to the human complement source to approximately 0.1mg/ml conc. Antibody-mediated C3c and C5b-9 deposition (ADCD) onto live N. gonorrhoeae was also determined.

Results

Mouse anti-OMV serum produced high bactericidal titres when tested against the homologous gonococcal strain. The addition of anti-C7 mAb blocked bactericidal killing. Greater killing titres were observed following the addition of differentiated HL60 cells at a ratio of 4:1 This greater killing was not reduced by addition of anti-C7 mAb. Addition of saponin to lyse the HL60s did not increase the CFU determined in OPA. No killing was observed with mouse anti-Bexsero serum. ADCD results were aligned to those obtained for SBA and OPA.

Conclusions

We have developed a range of tools that can be used to dissect antibody and complement-mediated immunity to the gonococcus for both laboratory animal and human sera.