Development of complement factor H based immunotherapeutic molecules against multidrug-resistant Neisseria gonorrhoeae

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*Neisseria gonorrhoeae* has become resistant to almost every antibiotic in clinical use. Novel therapeutics against this pathogen are urgently needed. Gonococcal lipooligosaccharide (LOS) often expresses lacto-N-neotetraose (LNnT), which becomes sialylated in vivo, enhances factor H (FH) binding, and contributes to the bacteria’s ability to resist killing by complement. We previously showed that FH domains 18–20 (with a D to G mutation at position 1119) fused to Fc (FHD1119G/Fc) activated complement. This killed sialylated gonococci in vitro and was efficacious against gonococci in mice. Gonococcal LOS can phase-vary because of slipped-strand mispairing of LOS glycosyltransferase (Igt) genes, causing the bacteria to lose the ability to express LNnT and results in diminished sialylation of LOS. Diminished LOS sialylation, although likely to be associated with a considerable fitness cost, could decrease efficacy of FHD1119G/Fc binding. Similar to *N. meningitidis*, gonococci also bind FH domains 6 and 7 through Neisseria surface protein A (NspA). A fusion protein comprising FH domains 6 and 7 fused to human IgG1 Fc, termed FH6,7/Fc, bound to all 15 wild-type gonococci tested and to each of six lgtA deletion mutants. FH6,7/Fc mediated complement-dependent killing of 8 out of 15 tested wild-type gonococcal strains and was as effective as FHD1119G/Fc in reducing the duration and burden of three gonococcal strains tested in a mouse vaginal colonization model, including two strains that resisted direct complement-dependent killing. FH6,7/Fc enhanced C3 deposition on both strains. FHD1119G/Fc and FH6,7/Fc were expressed in high yields in tobacco plants (550–650 mg/kg biomass post Protein A chromatography). The molecules produced in plants were as efficacious as CHO cell–expressed molecules in vitro and in vivo when administered as a topical intravaginally, thus offering an economical platform for product development. In summary, FH6,7/Fc and FHD1119G/Fc may represent promising prophylactic or adjunctive immunotherapeutics against multidrug-resistant gonococci. The use of these two FH/Fc molecules that target distinct Ng ligands could increase the breadth of Ng strain coverage and may overcome the potential of immune evasion by LOS phase variation.

**Keywords:** Factor H, Gonorrhea, Immunotherapeutics

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Chronic intradermal C5a exposure stimulates cardiac fibrosis – A role of inflamed lymphatic endothelium

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Primary and secondary lymphedema contribute significantly to host immune function in infective and non-infective endocarditis leading to heart failure, but are largely overlooked in terms of clinical interventions. Given that exacerbated inflammation via endothelial dysfunction is now viewed as a major cause of lymphatic disease, it is reasonable to propose C5a as a major molecular contributor to dysfunctional lymphatic endothelium and therefore to pathophysiology of endocarditis. To investigate this possibility, we examined if chronic intradermal C5a challenge (2.5 μg/40 μl at 24-h interval for 7 days) to C57B6/J mice would induce lymphatic endothelial inflammation and induce endocarditis. In transcriptome array analyses, endothelial activation by C5a was evident in inguinal lymph node tissue extracts, with significant upregulation of angiotensin II receptor, type 1a (AgIItr1a); cytokines and chemotactic factors Cx3Cl1, CxCl1, IL-3, Fas ligand (Fasl), and the clotting/angiogenic factors, Coagulation factor 3 (F3), Occludin (Ocln), Plasminogen (Plg), and thromidine phosphorylase (Tym). Additionally, reciprocal correlation was found between the upregulation of coagulation factors, F3, Ocln, and Plg with the downregulation of matrix molecules Kdr, Flt-1,serpine-1, thrombomodulin and Von Willebrand factor homolog. We also measured downregulation of angiotensin-1 converting enzyme 1 (Ace), the vasoconstriction regulator, endothelin receptor A (EdntrA), and the cardiac pressure-regulating natriuretic peptide hormone-binding receptor, atrionatriuretic peptide receptor 1 (Npr1). Similar to the inguinal lymph nodes, heart tissue extracts revealed upregulation of inflammatory transcripts, as well as a 2.4-fold upregulation of the vasoconstriction and blood pressure regulator, endothelin-2 (Edn2). In an experimental model of collagen type II-induced lymphedema, we measured in mouse hearts increases in expression levels of inflammation, fibrosis and remodeling markers, consistent with the results observed in the heart tissue of mice subjected to chronic intradermal C5a challenge. We confirmed the cardiac tissue inflammation and fibrosis with immunohasays. Together, our data demonstrate that C5a-induced lymphatic endothelial inflammation in lymphedema-lymph nodes correlates with cardiac inflammation and fibrosis, indicating that C5a may be a major mediator of lymphatic endothelial dysfunction in the development and pathophysiology of endocarditis.

**Keywords:** Endocarditis, Anaphylatoxin signaling, Lymphatic vasculature

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