Potentiation of efficacy of a candidate immunotherapeutic antibody to Neisseria gonorrhoeae by enhancing IgG Fc hexamer formation

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Neisseria gonorrhoeae (Ng), the causative agent of the sexually transmitted infection gonorrhea, has become multidrug-resistant. mAb 2C7 recognizes a gonococcal lipoooligosaccharide (LOS) epitope that is expressed by over 95% of gonococci in vivo and is targeted by a gonococcal vaccine candidate. We previously showed that murine mAb 2C7 attenuated gonococcal vaginal colonization in mice. A chimeric human IgG1 derivative of mAb 2C7, which contained the E430G Fc mutation at the CH2-CH3 interface to enhance IgG hexamerization and complement activation (Hexabody® technology), showed greater bactericidal activity against Ng. When the 2C7-E430G was administered intravaginally, a 5-fold lower dose cleared infection in wild-type mice compared to chimeric 2C7 with unmodified human IgG1 Fc. Gonococcal complement resistance is host-restricted because Ng selectively binds the human complement inhibitors, factor H (FH) and C4b-binding protein (C4BP). Accordingly, a 5-fold higher dose (than in wild-type mice) of intravaginal 2C7-E430G was required to clear gonococcal infection human FH/C4BP dual transgenic mice, where the mAb had to surmount the dampening effects of bacteria-bound complement inhibitors. A single 1 µg intravenous dose of 2C7-E430G significantly shortened the duration and decreased Ng burden in FH/C4BP transgenic mice. Chlamydia often co-infects with gonorrhea and increases the burden of Ng infection. 2C7-E430G was also effective against Ng in a chlamydia/Ng coinfection model. Based on studies in mice, several lines of evidence suggested that complement activation was necessary and sufficient for 2C7 function. First, complement-inactivating Fc mutations, but which permitted FcγR engagement, rendered 2C7 ineffective. Second, 2C7-E430G was non-functional in C1q−/− mice or when C5 function was blocked. Finally, 2C7-E430G function was maintained even after neutrophil depletion. These data identify complement activation as the mechanism of action of mAb 2C7 in vivo and validate the serum bactericidal assay as a correlate of Ab-mediated protection against gonorrhea. Our results illustrate the importance of complement for eradication of pathogens at the mucosal surface. Humanized 2C7 with enhanced ability to activate complement using Hexabody® technology represents a promising adjunctive anti-gonococcal immunotherapeutic.

Keywords: Neisseria gonorrhoeae, Hexabody®, Immunotherapeutic, Human IgG1 Fc mutation, FcγR, Complement activation

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Therapeutic efficacy of AAV-mediated factor H gene transfer in a murine model of lethal C3 glomerulopathy

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Factor H (FH) is plasma regulator of the alternative pathway (AP) of complement activation. It is composed of 20 short consensus repeat (SCR) domains and inhibits complement activation both in plasma and on the cell surface. Single nucleotide polymorphism and rare mutations in human FH are associated with age-related macular degeneration and severe kidney diseases including C3 glomerulopathy (C3G) and atypical hemolytic uremic syndrome. Recombinant FH or its engineered variants are potential therapeutic agents in AP complement-mediated diseases, but poor pharmacokinetics in vivo presents a challenging hurdle to their use in protein replacement therapy. Here we provide proof of concept for using AAV-based gene transfer to deliver persistent and therapeutically efficacious FH in a murine model of lethal C3G. Mice with FH and properdin double mutations (FHm/mP−/−) developed complement-driven C3G and most died from crescentic glomerulonephritis by 10–12 weeks. We engineered a mouse FH construct consisting of SCR domains 1–4, 6–8 and 19–20 (mFH1-4.6-8.19-20) and used adeno-associated virus (AAV)-mediated gene transfer to deliver its expression in FHm/mP−/− mice. Control AAV or mFH1-4.6-8.19-20-AAV was administered to 7-week old FHm/mP−/− mice by i.v. injection at 1012 or 1011 gene copies/mouse, i.e. by that age was already developed severe C3G. Treated mice were followed for 12 weeks and terminal studies were performed to assess C3G disease. All 10 mice treated with control AAV died within 6 weeks after treatment, whereas 9/10 mice treated with mFH1-4.6-8.19-20-AAV survived the 12-week experiment. High plasma levels of mFH1-4.6-8.19-20 were detected one week post AAV infection and its expression was sustained throughout the experimental period. mFH1-4.6-8.19-20-AAV treatment reversed established C3G disease in FHm/mP−/− mice as indicated by halted plasma C3 and factor B consumption, marked reduction in proteinuria, leukocyturia and hematuria, and essentially absent glomerular C3 deposition and largely normal kidney histology. Our data demonstrate that AAV-based gene transfer is a feasible approach to achieve sustained and therapeutically efficacious level of engineered FH for the treatment of complement-mediated diseases such as C3G.

Keywords: C3 glomerulopathy, AAV gene therapy, Complement inhibition, Factor H

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