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Review

Utilizing complement evasion strategies to design complement-based antibacterial immunotherapeutics: Lessons from the pathogenic *Neisseriae*

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ABSTRACT

Novel therapies are urgently needed to combat the global threat of multidrug-resistant pathogens. Complement forms an important arm of innate defenses against infections. In physiological conditions, complement activation is tightly controlled by soluble and membrane-associated complement inhibitors, but must be selectively activated on invading pathogens to facilitate microbial clearance. Many pathogens, including *Neisseria gonorrhoeae* and *N. meningitidis*, express glycans, including *N*-acetylneuraminic acid (Neu5Ac), that mimic host structures to evade host immunity. Neu5Ac is a negatively charged 9-cabon sugar that inhibits complement, in part by enhancing binding of the complement inhibitor factor H (FH) through C-terminal domains (19 and 20) on FH. Other microbes also bind FH, in most instances through FH domains 6 and 7 or 18–20. Here we describe two strategies to target complement activation on *Neisseriae*. First, microbial binding domains of FH were fused to IgG Fc to create FH18-20/Fc (binds gonococci) and FH6,7/Fc (binds meningococci). A point mutation in FH domain 19 eliminated hemolysis caused by unmodified FH18-20, but retained binding to gonococci. FH18-20/Fc and FH6,7/Fc mediated complement-dependent killing in vitro and showed efficacy in animal models of gonorrhea and meningococcal bacteremia, respectively. The second strategy utilized CMP-nonulosonate (CMP-NulO) analogs of sialic acid that were incorporated into LOS and prevented complement inhibition by physiologic CMP-Neu5Ac and resulted in attenuated gonococcal infection in mice. While studies to establish the safety of these agents are needed, enhancing complement activation on microbes may represent a promising strategy to treat antimicrobial resistant organisms.

Contents

Abbreviations: NulO, nonulosonate; LOS, lipooligosaccharide; LNnT, lacto-*N*-neotetraose; Lst, LOS sialyltransferase; FH, factor H; FB, factor B; FD, factor D; MASP-2, Mannan binding lectin associated serine protease 2; FHL-1, Factor H-like protein 1; FHR-1, Factor H-related protein 1; Por, Porin; NHS, normal human serum; SBA, serum bactericidal activity; NTHi, nontypeable *Haemophilus influenzae*.

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1. Introduction

Complement deficiencies have long been recognized as risk factors for certain infections (Figueroa et al., 1993; Figueroa and Densen, 1991; Ram et al., 2010) or as the cause of conditions such as paroxysmal nocturnal hemoglobinuria (PNH), for example, in which the loss of GPI-anchored membrane complement inhibitors CD55 and CD59 on erythrocytes leads to hemolysis (Nicholson-Weller et al., 1985; Pangburn et al., 1983). Over the past two decades, the role of complement dysregulation in various pathologic states has been recognized increasingly (de Cordoba et al., 2012; Hajishengallis et al., 2015; McHarg et al., 2015; Schramm et al., 2014; Thurman and Holers, 2006). Loss-of-function mutations in molecules that inhibit complement such as FH, membrane cofactor protein (MCP; CD46) and factor I (FI), or gain-of-function mutations in molecules that activate complement such as C3 and factor B (FB) all lead to an overactive alternative pathway and are associated with atypical hemolytic uremic syndrome (aHUS), a condition characterized by thrombotic microangiopathy and renal failure (de Cordoba and de Jorge, 2008; Esparza-Gordillo et al., 2005, 2006; Fremeaux-Bacchi et al., 2008; Hofer et al., 2014; Kavanagh and Goodship, 2010; Liszewski and Atkinson, 2015; Loirat and Fremeaux-Bacchi, 2011; Nester et al., 2015; Pickering and Cook, 2008). A common polymorphism in domain 7 of human FH (402H) reduces the ability of FH to bind to malondialdehydes in drusen (the retinal lesions seen in age-related macular degeneration), which is associated with increased alternative pathway activation and accelerated vision loss (Edwards et al., 2005; Haines et al., 2005; Klein et al., 2005; Weismann et al., 2011). Excessive complement activation may also play a role in neurological conditions such as Alzheimer's disease and schizophrenia (Hong et al., 2016; Sekar et al., 2016).

Complement-based therapeutics that are currently in clinical use or in pre-clinical trials all inhibit the complement cascade (reviewed in (Reis et al., 2015)). Purified C1 inhibitor is indicated for the treatment of hereditary or acquired C1 inhibitor deficiency. A humanized monoclonal antibody, eculizumab, has been used for several years to treat paroxysmal nocturnal hemoglobinuria (PNH) and more recently has been used successfully in several cases of shiga-toxin associated hemolytic uremic syndrome (Delmas et al., 2014; Dinh et al., 2015; Lapeyraque et al., 2011). Other products in various stages of development include: antibodies or fragments of antibodies directed against C5, factor D, C1s or MASP-2; small molecules that block factor D, C5aR or C3 or soluble complement inhibitors of complement (CR1 or fragments of FH). All these agents are being evaluated for a variety of conditions where complement inhibition may be beneficial (Reis et al., 2015).

In contrast to blocking the various complement pathways as described above, the goal of a complement-based anti-infective immunotherapeutic or prophylactic is to selectively activate the cascade on the microbial surface without causing collateral damage to normal host tissue. This is usually accomplished by molecules that specifically bind to invading pathogens and initiate complement activation. Antibodies that are elicited following natural infection or by immunization are historically the best appreciated initiators of the classical pathway, although the roles of lectins and ficolins in marking pathogens for subsequent complement activation are now well established (Degn and Thiel, 2013; Thiel and Gadjeva, 2009).

Immune antibodies are highly effective in preventing infections but their specificity can be a limitation. Extensive antigenic diversity even within a pathogenic species is a major challenge. As an example, over 90 distinct capsule types have been identified in *Streptococcus pneumoniae* (Kamerling, 2000), of which only 13 or 23 are targeted by conjugate or polysaccharide vaccines, respectively. Antigenic variation is a major hurdle in the development of vaccines against bacteria such as nontypeable *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Protective epitopes often are encoded by several alleles and/or expression these epitopes may be regulated by phase-variable genes (Barnett et al., 2015; Hill et al., 2010; Lipsitch and O'Hagan, 2007; Telford, 2008). Antibodies against more conserved epitopes sometimes are not broadly protective and may even be subversive ('blocking' antibodies) (Ray et al., 2011; Rice et al., 1986; Schweinle et al., 1989). Broad spectrum immunotherapeutics that target common pathogenic mechanism(s) across several pathogens would permit empiric treatment while awaiting a specific microbiologic diagnosis.

2. Mimicry of host glycans by pathogens

Several microbes evade host immunity by expressing glycans that mimic host sugars. Capsular polysaccharides produced by group B *N. meningitidis*, *Escherichia coli* K1, *Mannheimia haemolytica* and *Moraxella nonliquefaciens* all comprise α(2,8)-linked Neu5Ac, which is identical to human neural cell adhesion molecule (NCAM) (reviewed in (Cress et al., 2014)). *E. coli* K4, *Pasturella multocida* type F and *Avibacterium paragallinarum* (genotype I) all produce chondroitin sulfate capsules. Capsules containing heparosan are produced by *E. coli* K5, *P. multocida* (type D), *A. paragallinarum* (genotype II), *Streptococcus pyogenes, S. equi* ssp. *zooepidemicus, S. dysgalactiae* ssp. *equisimilis*, *S. uberis*, *S. equi* ssp. *equi*, *P. multocida* (type A) and *A. paragallinarum* (Cress et al., 2014).

Host-like glycans are also expressed by lipooligosaccharides (LOSs) of *N. gonorrhoeae, N. meningitidis, Campylobacter jejuni* and *H. influenzae* (Aspinall et al., 1994; Houliston et al., 2011; Mandrell and Apicella, 1993; Mandrell et al., 1988; Yuki et al., 2004,1993; Mandrell, 1992). Relevant to this review, two 'host-like' structures expressed by Neisserial LOS structures include lacto-*N*-neotetraose (LNnT; Galβ1-4GlcNAcβ1-3Galβ1-4Glc), identical to the terminal tetrasaccharide of paragloboside, a precursor of the major human blood group antigens (Mandrell et al., 1988), and globotriose (Galα1-4Galβ1-4Glc) that is identical to terminal globotriose trisaccharide of the P^K-like blood group antigen (Mandrell, 1992). The LNnT structure is found in eight LOS immunotypes of *N. meningitidis* (Tsai and Civin, 1991); the P^K-like structure is also referred to as the L1 immunotype. Host-like glycan structures expressed by microbes do not elicit robust antibody responses and therefore enable pathogens to evade the immune response.

3. The role of sialic acid in Neisserial complement evasion

In 1970, Ward et al. reported that gonococci recovered directly from male urethral secretions (not sub-passaged onto routine culture media) were fully resistant to killing by complement in normal human serum (NHS), a property termed serum resistance (Ward et al., 1970). However, even a single passage of most isolates on routine culture media resulted in serum sensitivity, which suggested that in vivo, gonococci acquired a host factor that conferred com-

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