Complement-Mediated Bactericidal Activity Against Escherichia Coli and Neisseria Meningitidis

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INTRODUCTION

The complement system is an enzymatic cascade of more than 30 proteins that is activated via the classical pathway (CP), lectin pathway (LP), or alternative pathway (AP) upon recognition of bacterial surface antigens. Activation of complement leads to the generation of biologically active fragments that play a critical role in defense against invasive microorganisms. Complement fragments opsonize pathogens, recruit and activate inflammatory cells at the site of complement activation, and act as the nidus for assembly of the lytic membrane attack complex.

Complement-directed therapeutic approaches are highly promising for diseases of complement dysregulation including C3 glomerulopathy (C3G). However, complement blockade can be accompanied by increased risk of bacterial infections, particularly meningococcal disease, as has been reported in patients treated with the terminal pathway complement inhibitor, ecuclizumab.

Methods

Reagents: Human whole blood was obtained with informed consent. Normal human serum (NHS), complement-depleted NHS, complement proteins, anti-human C5, and GVB buffer were purchased from Complement Technology, Inc. (Tyler, Texas, USA). Wieslab functional ELISA kit was purchased from Euro Diagnostica (Sweden). Escherichia coli (E. coli) strain DH5α was purchased from ThermoFisher Scientific (Waltham, MA, USA). Neisseria meningitidis (N. meningitidis) A (ATCC 13077) and N. meningitidis B (ATCC 700150) were purchased from ATCC. Phagotest kit was purchased from Glycotope Biotechnologie (Heidelberg, Germany). PE Mouse Anti-Human CD11b and PE-Cy7™ Mouse Anti-Human CD14 were purchased from BD Biosciences (San Jose, CA, USA).

Complement assays: NHS pre-incubated with or without ACH-4471 for 10 minutes was incubated in Wieslab assay microtiter strips according to the manufacturer's recommendations for terminal complement component blockage.

Complement activation was determined by optical absorbance.

Bactericidal activity: Briefly, NHS or complement depleted serum was pre-incubated with or without ACH-4471 for 10 minutes. E. coli was added and reactions were incubated at 37°C for 30 minutes. Reactions mixture was then stained with SYBR® Green I Nucleic Acid Stain and propidium iodide and bacterial viability was analyzed on BD Accuri C6 flow cytometer. Sera from healthy subjects with positive antibody titers against N. meningitidis were identified and used to evaluate ACH-4471, a C5 small molecule inhibitor of the AP pathway, versus factor D (BD) currently in clinical development for C3G and paroxysmal nocturnal hemoglobinuria (PNH; Table 1).

Using normal human serum, complement-depleted serum, and purified complement components, and using Escherichia coli as a model microorganism, we assessed the mechanism(s) by which complement enhances serum bactericidal activity (SBA) and opsonophagocytosis.

Additionally, using normal human serum, we evaluated the effects of ACH-4471 in assays of SBA activity against Neisseria meningitidis serogroups A and B.

Background & Results

Background on Complement System and Inhibitor Effects on AP & CP Activity and Meningococcal Killing

Fig. 1: Complement Pathways

Fig. 2: AP activity measured by Wieslab

Table 1: Effect of ACH-4471 and Eculizumab on the killing of meningococci by whole blood from vaccinated individual donors

Effect of Selective Complement Inhibition on SBA and Opsonophagocytosis against E. coli

Fig. 3: Serum Bactericidal Activity (SBA)

Fig. 4: Opsonophagocytosis

Effect of Selective Complement Inhibition on SBA against N. meningitidis

Fig. 5: Serum bactericidal activity (SBA) with ACH-4471 or CS blocking antibody

Effect of ACH-4471 on the killing of meningococci by whole blood from vaccinated individual donors

Conclusions

The terminal pathway is indispensable for bactericidal activity in vitro against Escherichia coli and Neisseria meningitidis.

Inhibition of AP by ACH-4471 caused no significant reduction in serum bactericidal activity and opsonophagocytosis of Escherichia coli in vitro.

ACH-4471 preserved serum bactericidal activity in vitro against Neisseria meningitidis in sera with anti-meningococcal antibody titers.

Lastly, ACH-4471 may be less likely than terminal pathway inhibitors including ecuclizumab to interfere with antibody-mediated protection against meningococcal and other bacterial infections.