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 on the surface of pathogens.
- 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, eculizumab.
- Recent studies have demonstrated that eculizumab impairs killing of meningococci by whole blood from immunized adults *in vitro*, whereas meningococcal killing is preserved in the presence of ACH-4471, an oral small-molecule inhibitor of the AP protease factor D (fD) 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.

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 700110) were purchased from ATCC. Phagotest kit was purchased from Glycotope Biotechnology (Heidelberg, Germany). PE Mouse Anti-Human CD11b and PE-Cy™7 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 complex formation. 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. Reaction mixtures were then stained with SYBR® Green I Nucleic Acid Gel 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 and a C5 blocking antibody in bactericidal assays with *N. meningitidis* A or B at 37°C for one hour. Colony forming units were quantitated following overnight growth on chocolate agar plates.
- **Monocyte and granulocyte isolation:** Heparinized blood from healthy donors was used for isolation of leukocytes via density gradient centrifugation. Cells were resuspended at 1x10^8 per ml in GVB^{++} buffer for phagocytosis assays.
- **Phagocytosis assay:** Briefly, NHS, complement-depleted NHS, or complement-depleted NHS reconstituted with the depleted complement protein(s) was pre-incubated with or without ACH-4471 for 10 minutes. Leukocytes and FITC-labeled *E. coli* were added and the reactions were incubated at 37°C for 15 minutes; in parallel a negative control reaction was incubated on ice. The final reactions consisted of 5 × 10^6 leukocytes and 40 × 10^6 FITC-labeled *E. coli*. Cells were labeled with PE mouse anti-human CD11b antibody and PE-Cy™7 mouse anti-human CD14 antibody and analyzed on BD Accuri C6 flow cytometer for identification of cell types.

BACKGROUND & RESULTS

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

Fig. 1: Complement Pathways

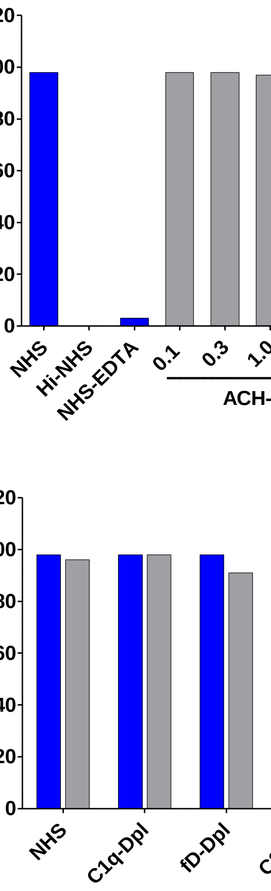

Fig. 2: AP activity measured by Wieslab

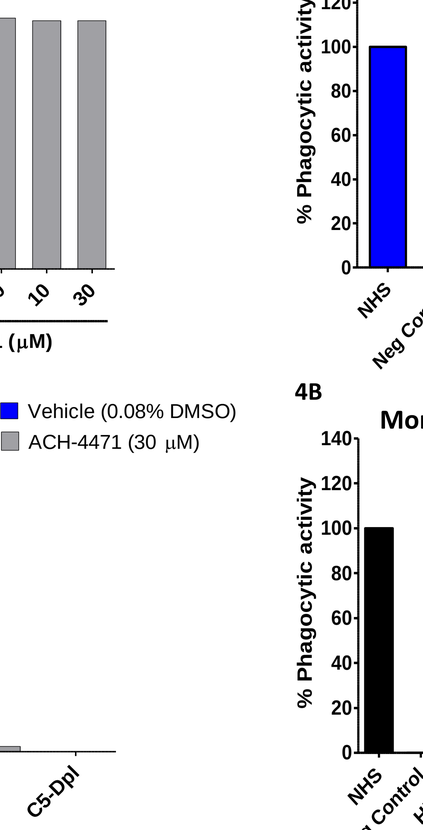

Table 1: Effect of ACH-4471 and Eculizumab on the killing of meningococci by whole blood from vaccinated individual donors

Subject ID	CFU/ml at 3 hr			
	Serogroup B, Strain H44/76		Serogroup C, Strain 4243	
	4 µM ACH-4471	50 µg/ml Eculizumab	4 µM ACH-4471	50 µg/ml Eculizumab
001	<100	131000	<100	60500
002	<100	65000	<100	112000
003	17800	92000	<100	79000
004	<100	231000	<100	237000
005	4800	126000	<100	98000
006	2900	45000	<100	105000
007	640	96000	5300	103000
008	14000	54000	25000	399000
009	<100	48000	<100	65000
010	<100	95000	<100	55500
011	<100	235000	<100	94500
012	<100	64100	<100	235000

<100 CFU/ml (sterile culture); <10 fold increase above control; No increase in CFU/ml above control; 10 to 100 fold increase above control

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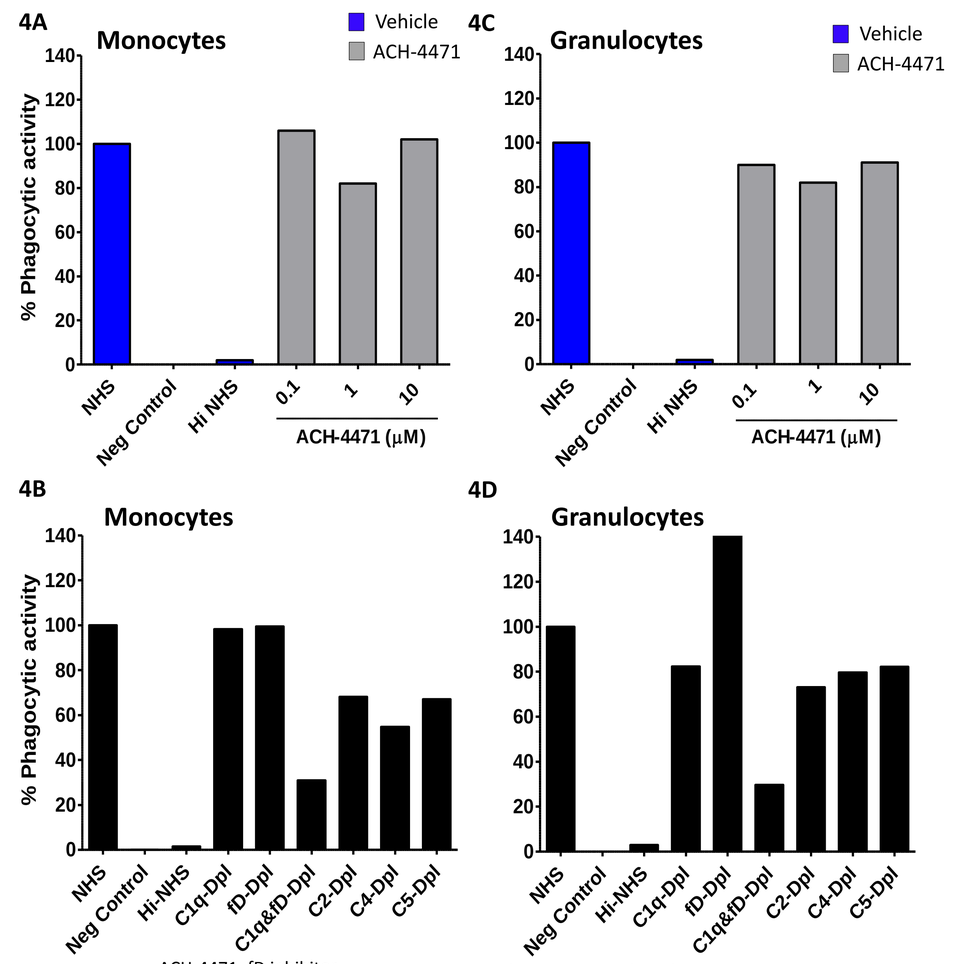
- **ACH-4471 potently and selectively inhibits complement AP activity**
- **ACH-4471 had significantly less inhibitory effect than Eculizumab on killing of meningococci by whole blood, especially when high titers of anti-meningococcal antibodies were maintained**

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

Fig. 3: Serum Bactericidal Activity (SBA)

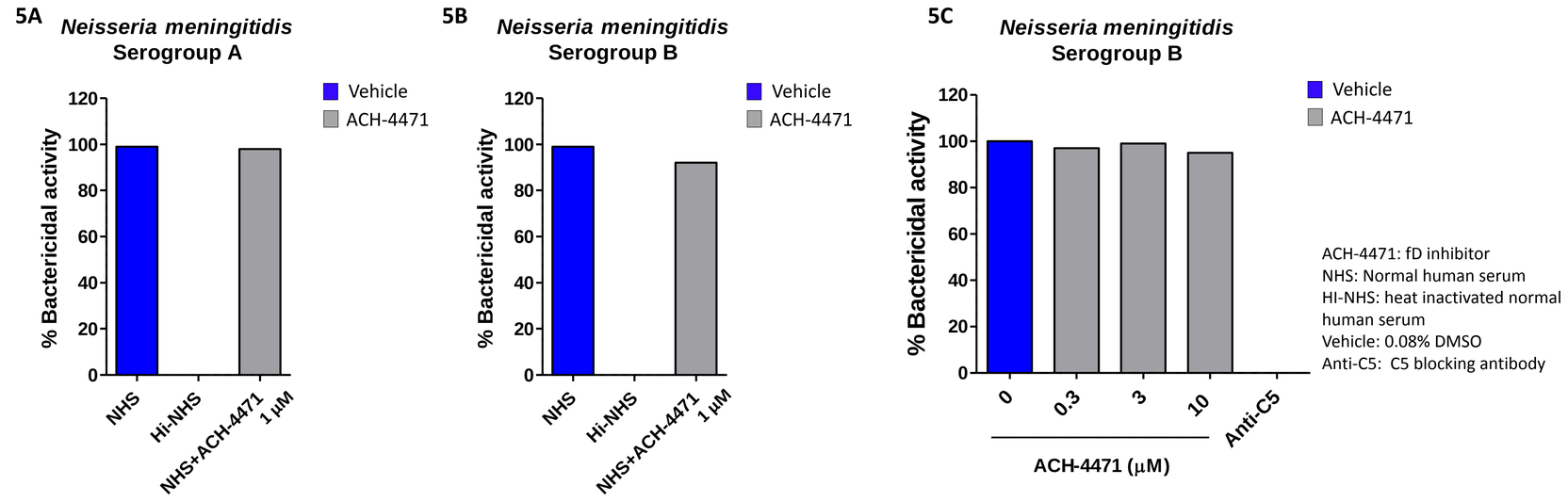
Fig. 4: Opsonophagocytosis



- **ACH-4471 did not alter bactericidal activity or opsonophagocytosis of *E. coli* by normal human serum**
- **There is significant redundancy in the contributions of complement pathways to bactericidal activity and opsonophagocytosis against *E. coli***
- **Blockade of all complement pathways (C2-Dpl + ACH-4471) or the terminal pathway (C5-Dpl) completely inhibited serum bactericidal activity**

Effect of Selective Complement Inhibition on SBA Against *N. meningitidis*

Fig. 5: Serum bactericidal activity (SBA) with ACH-4471 or C5 blocking antibody



- **ACH-4471 did not inhibit the bactericidal activity of normal human serum (NHS) against serogroup A and B test strains**
- **Blockade of the terminal pathway (C5 antibody) completely inhibited serum bactericidal activity against serogroup B strain**

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 eculizumab to interfere with antibody-mediated protection against meningococcal and other bacterial infections.**

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