The terminal complement pathway is indispensable for bactericidal activity against E. coli.

There is a significant degree of redundancy in the contribution of complement pathways for bactericidal activity and opsonophagocytosis of E. coli. Moreover, we evaluated the effects of factor D (fD) inhibitor ACH-4471 in the context of these experimental conditions.

ACH-4471 is a novel, highly potent and specific orally-administered small molecule inhibitor of fD, a serine protease with high homology to factor D.

ACH-4471 prevents factor B cleavage into Ba and Bb in the AP, leading to blockade of C3 convertase production and the Amplification loop upon activation of either the CP or LP.

ACH-4471 is currently in phase 1 clinical study.

RESULTS

Effect on Serum Complement Activities

Effect on Serum Bactericidal Activities

Effect on Opsonophagocytosis

Fig 1: AP Activity as Measured by Hemolysis and Wieslab

Fig 2: Bacterial Viability Measured in the Presence of NHS or Complement Depleted NHS

Fig 3: Opsonophagocytosis Measured in the Presence of NHS or Complement Depleted NHS

OBJECTIVES

• Assess the contribution of individual complement pathways to bactericidal activity and opsonophagocytosis in vitro

• Evaluate the effect of ACH-4471 on bactericidal activity and opsonophagocytosis in vitro

METHODS

• Reagents: Human whole blood was obtained with informed consent. Normal human serum (NHS), complement-depleted NHS, complement proteins, rabbit erythrocytes, FITC-labeled 40 µm E. coli, and FITC-labeled 60 µm E. coli were purchased from BD Biosciences (San Jose, CA USA). Wieslab assay was purchased from EuroDiagnostics, Sweden. Escherichia coli (E. coli) stain DH5α was purchased from Thermofisher Scientific (Waltham, MA USA). Phagocytosis kit was purchased from Glycotope Biotechnologies (Heidelberg, Germany). Reagents for 10 µM ACH-4471 and Pe-Cy5™ Mouse Anti-Human C1d were purchased from BD Biosciences (San Jose, CA USA).

• Complement assays: Briefly, NHS pre-incubated with ACH-4471 or vehicle control (DMSO) for 10 minutes was incubated with rabbit erythrocytes or in Wieslab assay microtiter strips according to the manufacturer’s recommendations for hemolysis activity or terminal complement complex formation, respectively. The degree of complement activation directly correlates with color intensity as measured by absorbance.

• Bactericidal activity: Briefly, NHS or complement-depleted serum was pre-incubated with ACH-4471 or vehicle control (DMSO) for 10 minutes. E. coli was added and then the reactions were incubated at 37 ºC for 30 minutes. At the end of the incubation period, the reaction mixtures were stained with SYBR® Green I Nucleic Acid Gel Stain and Propidium Iodide and viable bacteria analyzed on BD Accuri C6 flow cytometer.

• Monocyte and granulocyte isolation: Heparinized blood samples were used for isolation of leukocytes via density gradient centrifugation. Cells were resuspended at 1.4×10^6 per ml in FITC-labeled 40 µm E. coli.

• ACH-4471 and ACH-4471 + AP pathway inhibitor (CP or LP) completely inhibited serum bactericidal activity.

• Serum depleted of C2, C4 and C5 exhibited reduced phagocytic activity of monocytes and/or granulocytes (30 - 50% reduction).

• Serum depleted of C1q retained near complete phagocytic activity (<20% reduction) whereas depletion of both (C1q & fD-Dpl) exhibited a marked defect in phagocytic activity (~70% reduction). ACH-4471 did not alter the bactericidal activity of normal human serum (NHS).

• Serum depleted of C1q (CP), C2 (CP & LP) or ID (AP) retained complete bactericidal activity.

CONCLUSIONS

The terminal complement pathway is indispensable for bactericidal activity against E. coli. There is a significant degree of redundancy in the contribution of complement pathways for bactericidal activity and opsonophagocytosis.

Inhibition of ID by ACH-4471 did not significantly alter the bactericidal activity as well as monocyte/granulocyte opsonophagocytosis against E. coli at the concentrations that completely inhibited AP activity.

Clinical use of a ID inhibitor might present reduced infection risk compared to terminal complement pathway-inhibitors, especially in the population who have developed antibodies towards pathogens naturally and/or via vaccination.

Future studies are aimed at investigating other pathogens including bacteria that cause meningococcal disease.