Effect of Complement Inhibitors on Anti-meningococcal Plasma Bactericidal Activity and Whole Blood Opsonophagocytic Bactericidal Activity

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Background

Complement-directed therapeutic approaches are highly promising for diseases of complement dysregulation including paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. Complement can be activated by three independent pathways: classical pathway (CP), alternative pathway (AP), and lectin pathway. Complement activation via all three pathways results in cleavage of C3 and C5 and deposition of the activated fragments C3b and C5b, leading to assembly of the lytic membrane attack complex (MAC, C5b-9). People with complement deficiencies (acquired or inherited) have increased risk of infectious diseases. Because complement is essential in protection against meningococcal disease, the risk of disease in persons lacking a functional MAC is up to 1000-fold higher than in the general population. Further there are reports of cases of meningococcal disease in patients treated with eculizumab (Soliris), a humanized anti-C5 monoclonal Ab (mAb) that blocks cleavage of C5 to C5a and C5b. To mitigate this risk, the patients are routinely vaccinated against meningococci and it is thought that in the absence of MAC, protection is afforded by Fc-mediated opsonophagocytic activity (OPA) together with CP and AP activated production of additional opsonins, C4b and C3b (Figure 1). Eculizumab, however, also blocks generation of C5a, which is a potent chemotactic factor and up-regulator of receptors on phagocytic cells, including Fc receptor and the complement receptor CR3. The consequences of blocking both C5a generation and MAC assembly on OPA protection against meningococcal disease is not known. In addition, while protection afforded by vaccination relies on antibodies and CP activation, it is unknown whether blocking AP also will decrease protection, especially when antibody binding to the bacterial surface is limited because of low serum antibody levels and/or sparse antigen, when amplification of activated C3b and C5b fragments via the AP may be needed. To address these questions, we obtained plasma and whole blood samples from vaccinated individuals and assessed plasma complement-mediated bactericidal activity (BA) and killing of meningococci by OPA in the presence of eculizumab, which blocks both C5a and MAC, or ACH-4471, an oral inhibitor of complement factor D that specifically blocks AP.

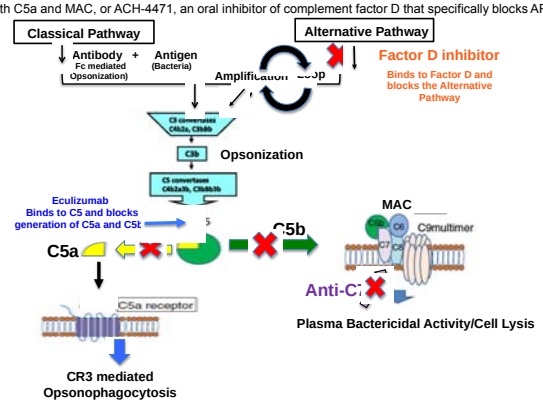

Figure 1. Model by which the CP and AP mediate anti-meningococcal BA and OPA. Blocking C5 or C7 in the terminal complement pathway prevents formation of MAC, which is required for complement-mediated BA. In the absence of MAC, anti-meningococcal antibodies can still protect by OPA, which involves phagocytic uptake and killing of opsonized bacteria via antibody-Fc and iC3b-CR3 receptor mechanisms. Blocking cleavage of C5 to C5a by eculizumab could reduce OPA by blocking chemotaxis and inhibiting up-regulation of phagocytic receptors such as CR3.

Aim

To investigate the effect of blocking C5 cleavage by Eculizumab, or inhibition of the AP by ACH-4471, on complement-mediated bactericidal activity (BA) and whole blood killing of meningococci (OPA) in healthy adults immunized with meningococcal vaccines

Methods

Fresh whole blood and plasma, both anticoagulated with lepirudin (recombinant hirudin, which inhibits thrombin), were obtained from 10 healthy microbiologists or hospital employees. The subjects had been immunized with two or three doses of a meningococcal serogroup B vaccine, either Trumenba (Pfizer, subjects 001 through 005), or Bexsero (GSK, subjects 006 through 010). The final dose was given 3 to 9 months prior to this study. All 10 subjects were also immunized 8 to 97 months earlier with a quadrivalent meningococcal A ,C, Y, W conjugate vaccine.

At time 0, ~ 5 X 10^3 CFU per ml of log-phase bacteria were added to whole blood or to a 1:4 dilution of plasma in the presence or absence of different complement inhibitors (Table 1). The inhibitor concentrations were in excess of those needed to block completely the respective complement pathway. Aliquots of whole blood or plasma were cultured following 1 or 3 hrs incubation to ascertain surviving CFU/ml of bacteria. We tested two reference strains, serogroup C 4243 and serogroup B H44/76, which have been used in previous studies to assess serum complement-mediated BA responses to meningococcal conjugate and serogroup B vaccines, respectively. In some experiments we also tested two additional serogroup B strains, which are known to be more resistant to vaccine-induced BA.

Table 1. Complement inhibitors used in study

Inhibitor	Concentration	Function
ACH-4471 (small molecule) Factor D Inhibitor	1 to 4 μM	Inhibits Factor D protease; blocks specifically the alternative pathway
Eculizumab (Anti -C5)	50 μg/ml	Binds C5: blocks the terminal pathway/MAC assembly and C5a release (chemotaxis and upregulation of phagocytic cell receptors)
Anti-C7 mAb	200 μg/ml	Binds C7: blocks the terminal pathway/MAC assembly
C5a receptor antagonist (small molecule)	10 μM	Binds C5aR: Inhibits C5a functions (chemotaxis and upregulation of phagocytic cell receptors)

Results

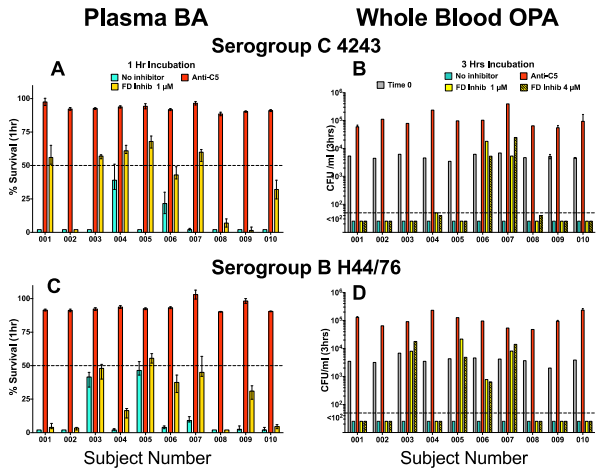

Figure 2. The effect of inhibition of C5 or Factor D on survival of meningococcal serogroup C and B test strains. Panels A and C, complement-mediated BA. Percent survival after 1 hr incubation of bacteria in plasma diluted 1:4. Horizontal line represents 50% survival compared to CFU/ml in negative control (bacteria growing in the presence of active complement with no bactericidal activity against the strain) at 1 hr. **Panels B and D, OPA bacterial killing.** CFU/ml after incubation of bacteria for 3 hrs in anticoagulated whole blood. Gray bars, CFU/ml added to blood at time 0. Horizontal line represents no survival compared to CFU/ml added at time 0.
In the absence of inhibitor, plasma or whole blood from all 10 subjects killed both strains. The addition of eculizumab (anti-C5) completely blocked killing in both plasma and whole blood. Blocking the AP partially inhibited plasma BA of the group C strain in 7 subjects and OPA killing in 2 subjects. Similar results were seen with the group B strain (3 and 4 subjects, respectively)

Table 2. Summary of whole blood OPA killing of meningococci by individual subject

Subject ID	CFU/ml at 3 hr			
	Serogroup B		Serogroup C	
	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	640	96000	5300	103000
007	14000	54000	25000	399000
008	<100	48000	<100	65000
009	<100	95000	<100	55500
010	<100	235000	<100	94500

<100 CFU/ml (sterile culture)
No increase in CFU/ml as compared to T_0
<5-fold increase in CFU/ml as compared to T_0
10 to 100-fold increase in CFU/ml as compared to T_0

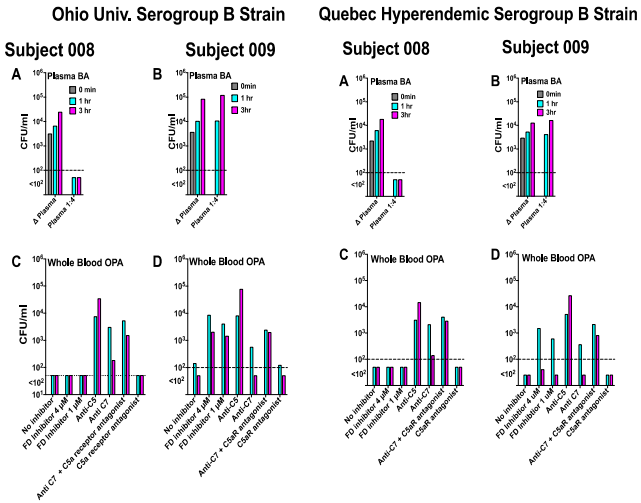

Figure 3. Effect of complement inhibitors on plasma and whole-blood killing activity of two subjects vaccinated with MenB-4C (Bexsero) against two clinical serogroup B strains: one that caused an outbreak at Ohio University (13 cases), and one that caused a hyperendemic disease in Quebec Canada. In previous studies both strains were more resistant to vaccine-induced serum BA than strain H44/76 tested in experiments shown in Figure 2. To separate the multiple activities blocked by eculizumab, anti-C7 (MAC only) and anti-C5a receptor (OPA only) were also tested individually and together. Δ Plasma=heat inactivated plasma Plasma alone (1:4) from subject 008 was sufficient for killing of both strains but plasma from subject 009 lacked bactericidal activity.

In the absence of inhibitor, whole blood from both subjects killed both strains after 1 to 3 hrs incubation. The addition of eculizumab completely blocked whole blood killing. Inhibiting the AP had no effect on whole blood killing of subject 008, but blocked or partially blocked bacterial killing by blood from subject 009.

Inhibition of C7 by an anti-C7 mAb, which blocks MAC formation and BA (similar to anti-C5) but does not affect release of C5a (Figure 1), had much less effect on blocking whole blood killing of both subjects compared with inhibition of C5 (particularly after 3 hrs incubation). The addition of a C5a receptor antagonist together with the anti-C7 mAb, blocked whole blood killing of both strains more than the anti-C7 alone. Thus, when MAC formation is blocked, C5a activity appears to be essential for optimal whole blood OPA.

Summary & Conclusions

1. **In the absence of inhibitor, plasma 1:4 or whole blood from 10 vaccinated subjects killed serogroup B and C reference strains**
2. **Whole blood from two subjects tested killed both the Quebec hyper endemic strain and Ohio Univ. serogroup B outbreak strain**
3. **Eculizumab completely inhibited whole blood opsonophagocytic killing of all strains in all subjects tested**
4. **Whole blood killing was observed when MAC assembly was blocked by anti-C7 mAb. When C5a activity also was blocked with a C5a receptor antagonist, the combination inhibited whole blood killing to a similar extent as eculizumab. Therefore the blocking of OPA whole blood killing by eculizumab resulted from blocking both MAC assembly and C5a-mediated chemotaxis and/or up-regulation of phagocytic complement receptors**
5. **Inhibition of AP with ACH-4471 had significantly less inhibitory effect than eculizumab on killing of meningococci by plasma or whole blood**
6. **Collectively, the efficacy of meningococcal vaccines is greatly impaired by eculizumab and is much less impaired by ACH-4471, especially when high levels of bactericidal and opsonic antibodies are maintained**

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