Differential effects of therapeutic complement inhibitors on serum bactericidal activity against non-groupable meningococcal isolates recovered from patients treated with eculizumab

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Supplemental eTable 1. Reports of meningococcal disease in US patients treated with eculizumab, January 2008–July 2018[†]

Case[#]	Year of onset	Disease*	Antibiotic prophylaxis	MenB vaccination status[&]	Diagnosis	Interval since starting Eculizumab	Interval since last MenB dose	Outcome	Serogroup**	Penicillin susceptibility[δ]	Ref
1[†]	2008	PNH	Unknown	N/A	Bacteremia	Unknown	N/A	Survived	Y	Sensitive	(1, 2)
2	2010	PNH	None	N/A	Bacteremia and meningitis	Unknown	N/A	Survived	NG	Sensitive	(1)
3	2010	Devic's disease	None	N/A	Bacteremia	2 months	N/A	Survived	NG	Sensitive	(1)
4	2011	PNH	None	N/A	Bacteremia	6 years	N/A	Survived	Not done[§]	Not done[§]	(1)
5	2011	PNH	None	N/A	Bacteremia	Unknown	N/A	Survived	Y	Sensitive	(1)
6	2011	PNH	None	N/A	Bacteremia	1 month	N/A	Survived	NG	Sensitive	(1)
7	2012	aHUS	None	N/A	Bacteremia	<10 months	N/A	Survived	NG	Sensitive	(1)
8	2012	PNH	None	N/A	Bacteremia and meningitis	Unknown	N/A	Survived	NG	Sensitive	(1)
9[†]	2014	PNH	None	N/A	Septic shock	2 years	N/A	Survived	NG	Sensitive	(1, 3)
10[†]	2015	PNH	Unknown	N/A	Septic shock	3 years	N/A	Survived	NG***	Intermediate	(1, 4)
11	2015	aHUS	Penicillin	N/A	Bacteremia and meningitis	2 months	N/A	Survived	NG	Intermediate	(1)
12	2015	aHUS	Unknown	N/A	Bacteremia	Unknown	N/A	Survived	Y	Resistant	(1)
13[†]	2016	PNH	None	MenB-4C (2 doses)	Septic shock	8 days	6 months	Died	NG	Sensitive	(1, 5)
14	2016	PNH	Unknown	None	Bacteremia and meningitis	Unknown	N/A	Survived	NG	Not done[§]	(1)
15	2016	aHUS	No	MenB-4C (2 doses)	Bacteremia	2 years	10 months	Survived	Y	Sensitive	(1)
16	2016	aHUS	No	MenB-4C (1 dose)	Bacteremia	2 years	15 months	Survived	NG	Intermediate	(1)
17	2018	PNH	No	None	Bacteremia	Unknown	N/A	Survived	NG	Intermediate	Not Reported
18	2018	PNH	Penicillin	MenB-4C (2 doses)	Bacteremia	7 months	6 months	Survived	NG	Intermediate	Not Reported

[#]Isolates from cases shaded in grey are further analyzed in the main text of the paper.

[†]Cases 1, 9, 10 and 13 were reported by MacNeil et al [2], Applegate et al [4], Hawkins et al [3] and Nolfi-Donegan et al [5], respectively, and are included in the 16 cases reviewed by McNamara et al [1].

*PNH, paroxysmal nocturnal hemoglobinuria; aHUS, atypical hemolytic uremic syndrome; Devic's disease or Devic's syndrome is also known as neuromyelitis optica.

[&]16 patients had documentation of a quadrivalent serogroup A, C, Y and W polysaccharide or conjugate vaccine prior to meningococcal disease onset. MenB-4C, serogroup B vaccine (Bexsero®, GSK); N/A, not applicable (onset of meningococcal disease was before MenB vaccination was available or recommended, see text).
**Serogroup determined by slide agglutination in combination with sequencing of the capsular polysaccharide locus except for case 14, where serogroup was determined via PCR on a clinical specimen (no isolate available). NG, nongroupable (also signifies not encapsulated)
§Not done because no specimen (case 4) or no isolate (case 14) was sent to CDC
***Serogroup originally reported as "indeterminate", and determined to be NG after retesting at CDC.
[δ]Susceptibility to penicillin was measured by reference broth microdilution. Susceptible strains were defined as MIC ≤0.06 μg/ml; intermediate susceptibility,
0.12 to 0.25 μg/ml and resistant strains, ≥ 0.5 μg/ml

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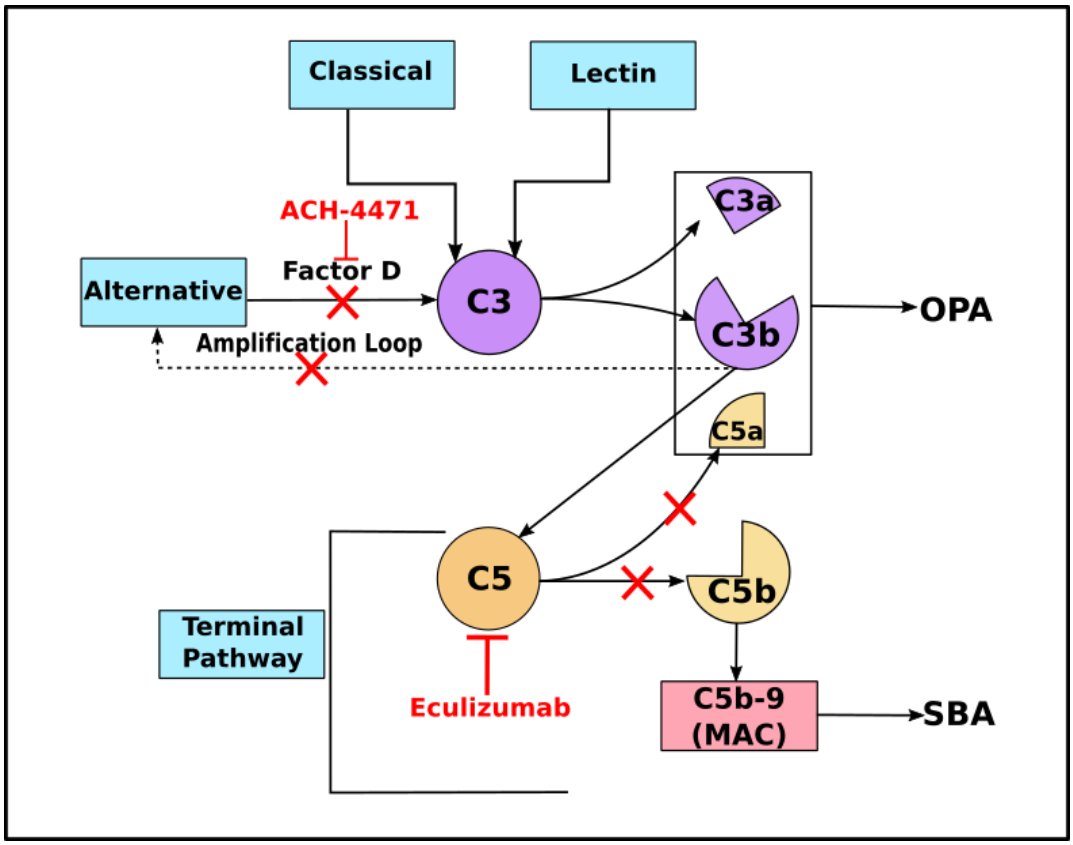
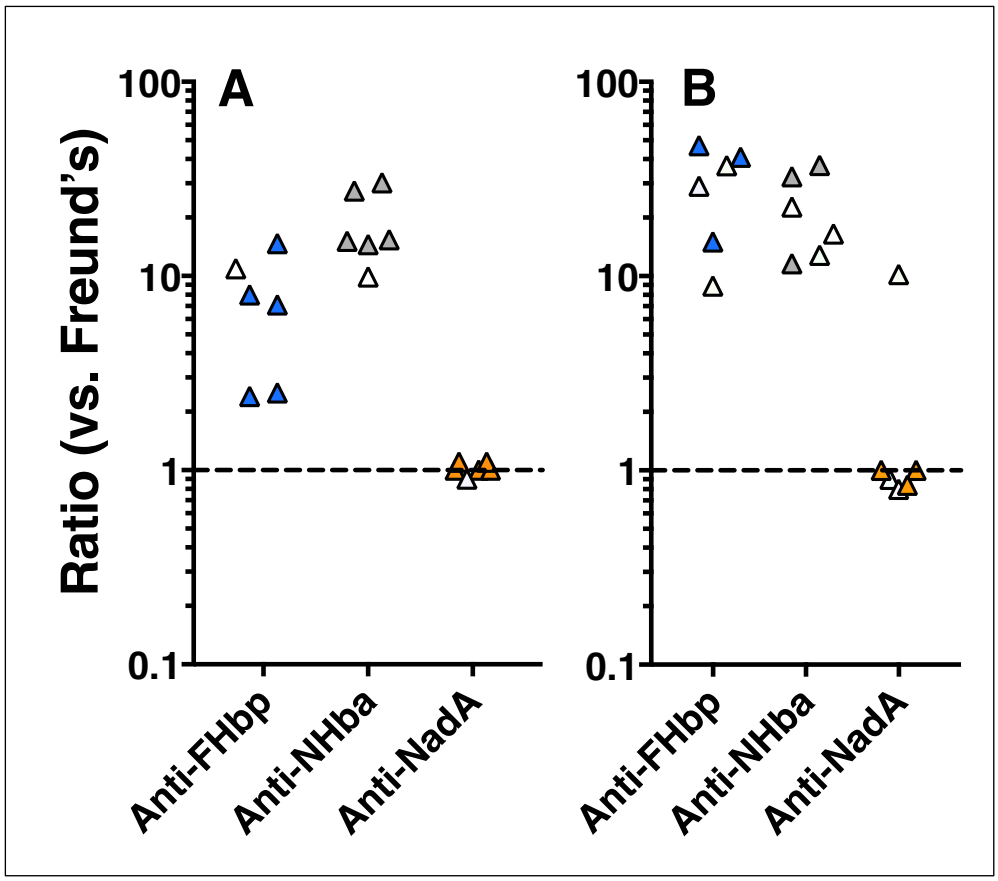
Supplemental eTable 2. Summary of the effect of different complement inhibitors on SBA of healthy donors (8 vaccinated and 8 unvaccinated) measured against a control serogroup B encapsulated strain and 8 NG meningococcal isolates from patients receiving treatment with eculizumab who developed invasive meningococcal disease

Strain	Inhibitor	No. of Serum Samples with Bacterial Survival >50% / No. Serum Samples Tested			
		at 1:5 Serum Dilution		at 1:2.5 Serum Dilution	
		Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
H44/76 Capsular B	None	**8 / 8**	0 / 8	Not Done	Not Done
	Eculizumab	8 / 8	8 / 8		
	ACH-4471	8 / 8	0 / 8		
CH891 NG, Disrupted B Capsular Locus	None	0 / 8	0 / 8		
	Eculizumab	8 / 8	8 / 8		
	ACH-4471	0 / 8	0 / 8		
CH892 NG, B Capsular Phase Variable Off	None	0 / 8	0 / 8		
	Eculizumab	8 / 8	8 / 8		
	ACH-4471	0 / 8	0 / 8		
CH897 NG, Disrupted Y Capsular Locus	None	0 / 8	0 / 8		
	Eculizumab	8 / 8	8 / 8		
	ACH-4471	0 / 8	0 / 8		
CH894 NG, Disrupted Y Capsular Locus	None	0 / 8	0 / 8		
	Eculizumab	8 / 8	8 / 8		
	ACH-4471	0 / 8	0 / 8		
CH893 NG, Disrupted Y Capsular Locus	None	0 / 8	0 / 8		
	Eculizumab	8 / 8	8 / 8		
	ACH-4471	**5 / 8**	**1 / 8**		
CH885 NG, Capsular Null	None	0 / 8	0 / 8		
	Eculizumab	8 / 8	8 / 8		
	ACH-4471	0 / 8	0 / 8		
CH896 NG, Capsular Null	None	0 / 8	0 / 8		
	Eculizumab	8 / 8	8 / 8		
	ACH-4471	**1 / 8**	0 / 8		
CH886* NG, Disrupted E Capsular Locus	None	**2 / 8**	0 / 8	**0 / 6**	0 / 3
	Eculizumab	8 / 8	8 / 8	6 / 6	3 / 3
	ACH-4471	**8 / 8**	**3 / 8**	**6 / 6**	**0 / 3**

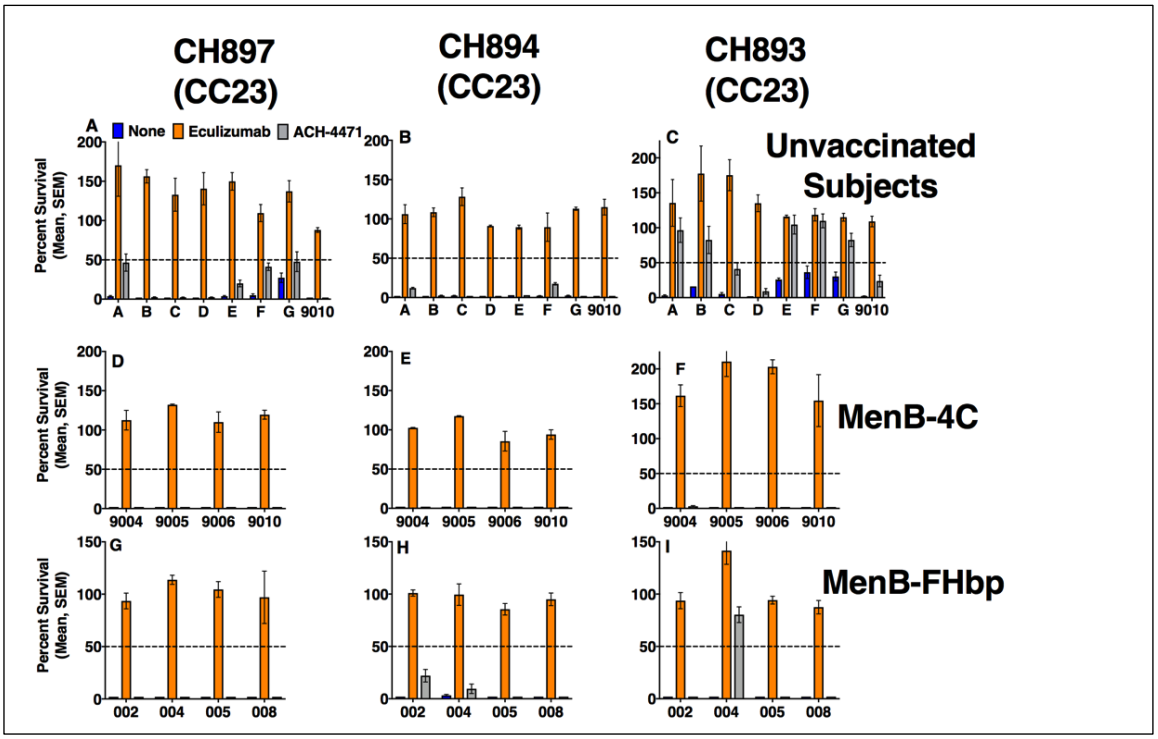
Note: A positive SBA required ≤50% survival of bacteria after incubation of bacteria at 37° C for 60 mins. Cells highlighted with shading represent data that was discordant with the respective results for the majority of the test isolates and sera.
*For strain CH886, 6 of the 8 unvaccinated sera gave ≤50% bacterial survival when tested at a 1:5 dilution in the absence of inhibitor (i.e., SBA titers ≥1:5). At a 1:5 or 1:2.5 serum dilution, SBA was inhibited in all six with 1 μM or 2 μM ACH-4471, respectively. All 8 vaccinated sera had SBA titers ≥1:5 in the absence of inhibitor. At a serum dilution of 1:5, SBA was inhibited by 1 μM ACH in 3 of the 8 vaccinated sera but when the three sera were retested at a dilution of 1:2.5 with 2 μM ACH-4471, there was ≤50 bacterial survival for all three. At both dilutions SBA was inhibited by 50 μg/ml of eculizumab.



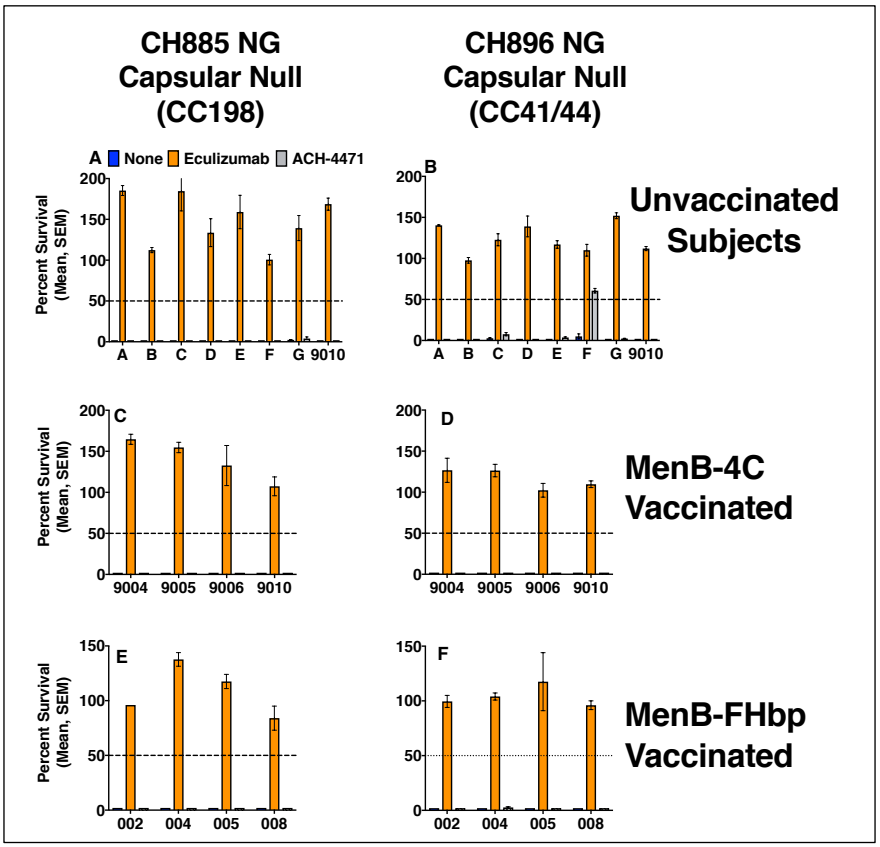
Supplemental eFigure 1. Schematic of the complement pathways. Activation of the classical complement activation pathway (CP) by antibodies, or of the lectin (LP) or alternative (AP) pathways, leads to C3 cleavage to C3a and C3b. Sufficient C3b deposition on the bacteria can activate the terminal complement pathway (C5 to C9) with formation of a functional C5b-9 membrane attack complex (MAC) required for meningococcal serum bactericidal activity (SBA). Eculizumab blocks cleavage of C5 to C5a and C5b. In the absence of C5b, the MAC cannot form and SBA is blocked. Release of C5a also is blocked, which impairs opsonophagocytic (OPA) killing of meningococci (6). ACH-4471 binds to Factor D, inhibiting the AP. When the AP is blocked, the terminal pathway remains intact and can be activated by specific antibodies via the CP, which permits SBA. The AP amplification loop is needed for SBA when there is insufficient C3b deposition for formation of MAC by CP (or LP) alone. Note that when the AP is blocked by ACH-4471, C5a-dependent opsonophagocytic killing also can proceed via CP (or LP) even when SBA alone is insufficient for killing.



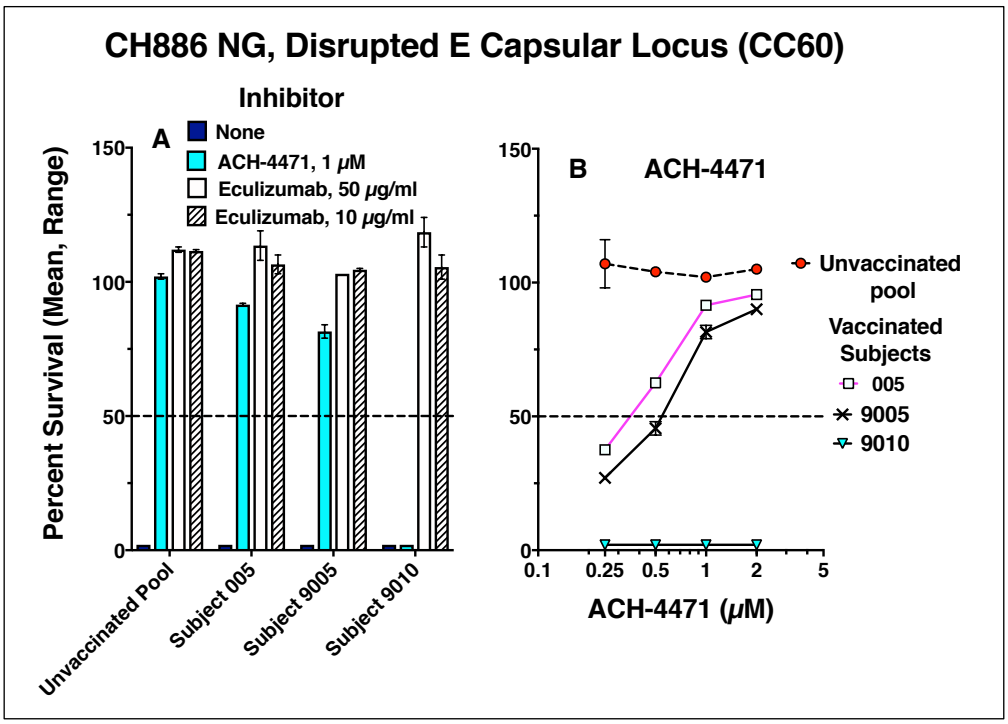
Supplemental eFigure 2. Reactivity of anti-FHbp, anti-NHba and anti-NadA antibodies to the 12 available nongroupable meningococcal isolates from patients treated with eculizumab as measured by flow cytometry. Panel A, isolates with FHbp sub-family A (N = 6). Panel B, isolates with FHbp sub-family B (N = 6). All antisera were tested at a 1:500 dilution. Each data point represents the ratio of the median binding florescence measured with a specific antiserum compared to that of a negative control serum pool from mice immunized with Freund's adjuvant without antigen. The antigen binding by the four isolates not tested in the SBA analysis is represented by open symbols (1 isolate in Panel A and 3 in Panel B; see text).



Supplemental eFigue 3. Effect of complement inhibitors on serum bactericidal activity against three NG isolates with disrupted Y capsular loci. All three strains are from CC23, have identical FHbp, NHba, and PorA VR types, and lack NadA genes (Table 1). The horizontal line indicates 50% survival of bacteria after 60 minutes incubation at 37°C. Blue bars, test sera with no added inhibitor; orange bars, test sera with 50 µg/ml eculizumab; and black bars, test sera with 1 µM ACH-4471. Panels A–C, unvaccinated adults; Panels D–F, adults vaccinated with 2 doses of MenB-4C with last dose given 1 to 11 months earlier; Panels G–I, adults vaccinated with 3 doses of MenB-FHbp with last dose given 6 to 11 months earlier. Blocking the terminal pathway with eculizumab completely blocks SBA while blocking the AP with ACH-4471 had minimal or no effect on decreasing SBA against isolates CH897 and CH894. However, against isolate CH893, SBA was inhibited for 5 of 8 unvaccinated subjects and 1 of 8 vaccinated subjects. Data for each isolate are from 2 to 4 replicate assays.



Supplemental eFigure 4. Effect of blocking C5 or Factor D on serum bactericidal activity measured against two capsular null NG isolates. Both isolates expressed NHba (present in MenB-4C) and FHbp subfamily B (present in MenB-4C and MenB-FHbp). All sera were diluted 1:5 and assayed with internal (endogenous) complement. CH885 is from CC198 and CH896 is from hypervirulent lineage CC41/44 . The horizontal line indicates 50% survival of bacteria after 60 minutes incubation at 37°C. Blue bars, test sera with no added inhibitor; orange bars, test sera with 50 µg/ml eculizumab; and black bars, test sera with 1 µM ACH-4471. Panels A and B, unvaccinated adults; Panel C and D, adults vaccinated with 2 doses of MenB-4C with last dose given 1 to 11 months earlier; Panels E and F, adults vaccinated with 3 doses of MenB-FHbp with last dose given 6 to 11 months earlier. Only one unvaccinated subject (F) had a titer <1:5 against isolate CH896 when the AP was blocked by ACH-4471 (Panel B; bacterial survival >50%). Data are from 2 to 4 replicate assays.



Supplemental eFigure 5. Concentration-dependent inhibition of SBA against NG isolate CH886. A. Eculizumab, 10 or 50 µg/ml. Eculizumab gave complete inhibition of SBA by unvaccinated or vaccinated sera at both inhibitor concentrations. ACH-4471 (1 µM) inhibited SBA of the unvaccinated serum pool and vaccinated sera from subjects 005 and 9005 but not subject 9010. **B. ACH-4471, 0.25 to 2 µM**. As little as 0.25 µM ACH-4471 completely inhbited SBA of the unvaccinated serum pool whereas there was no inhibition of SBA of vaccinated subject 9010 even by 2 µM ACH-4471. SBA of vaccinated subjects 9005 and 9010 showed concentration-dependent inhibition by ACH-4471 from 0.25 to 2 µM.