

The Role of anti-NHba Antibody in Bactericidal Activity Elicited by the MenB-4C Vaccine



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Abstract

Background. MenB-4C contains four antigens reported to elicit serum bactericidal activity (SBA). The bactericidal role of antibodies to NHba, or cross-reactive antibodies to FHbp sub-family A is poorly understood.

Methods. We selected sera from 9 adults with ≥ 3 -fold increases in SBA titer against a strain that expresses NHba (100% amino acid identity to vaccine) but is antigenically mismatched for the other three MenB-4C antigens. Post-immunization sera were depleted of antibodies to NHba and/or FHbp by column adsorption.

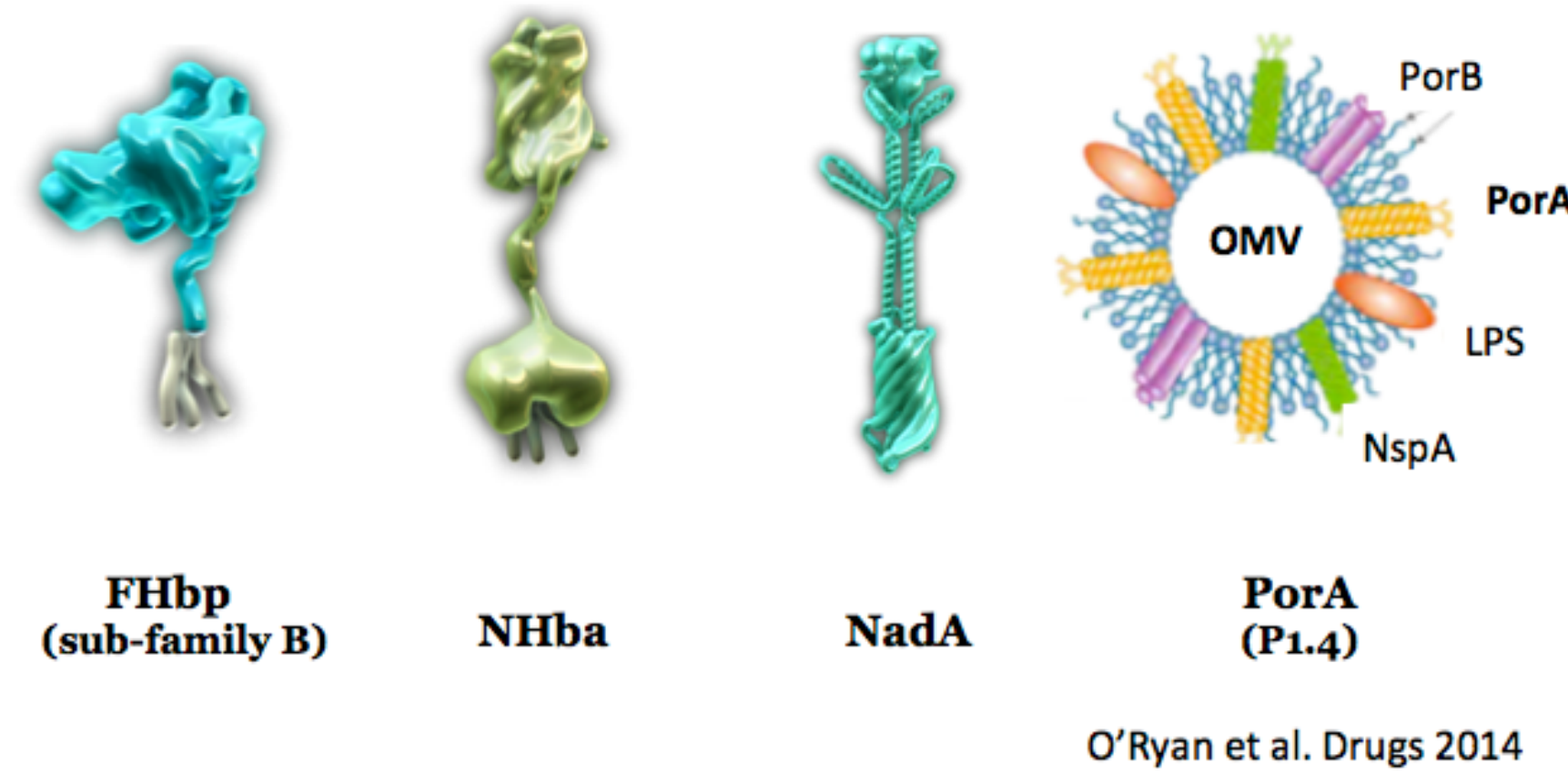
Results: Against three strains with FHbp sub-family A (mismatched with the vaccine) and matched only for NHba, depletion of anti-FHbp antibodies decreased SBA by $\geq 50\%$ in 2 to 8 sera depending on the strain. Depletion of anti-NHba also decreased titers in 3 to 6 sera. Against a fourth strain mismatched for all 4 vaccine antigens, depletion of anti-FHbp antibody decreased SBA (mean of 100% for six sera). Against a strain matched for sub-family B FHbp and NHba (100% identity to vaccine), depletion of anti-FHbp antibody decreased SBA titers (mean=92%), whereas depletion of NHba antibody had a small effect (mean =26%).

Conclusions. Antibodies elicited by the sub-family B FHbp vaccine antigen contribute to SBA against strains with FHbp sub-family A. Anti-NHba antibodies also contribute to SBA. However, some strains are resistant to anti-NHba antibody, other strains require antibodies to second antigens such as FHbp to elicit anti-NHba SBA.

Background

- MenB-4C contains 4 antigens (Figure 1) reported to contribute to complement-mediated serum bactericidal activity (SBA), which is an accepted correlate of protection
- For vaccine licensure, FHbp, NadA and PorA were shown to contribute to protection
- In humans, the role of antibodies to the fourth antigen, NHba, in SBA is remains undefined

Figure 1. MenB-4C (Bexsero, GSK)



O'Ryan et al. Drugs 2014

Methods

Selection of Sera

- 9 subjects were selected based on ≥ 3 -fold increase in serum bactericidal titer (comparing post titers to pre-immunization titers, Figure 2, Panel A)
- The test strain (B1) has high expression of NHba (100% amino acid identity to vaccine) and is mismatched for the other three MenB-4C antigens (FHbp subfamily A, absent NadA gene, PorA other than P1.4)
- Sera were tested against 6 additional strains (Table 1).
- Figure 2, Panel B, shows the number of subjects tested against each strain (based on 3-fold or greater increases in titer)

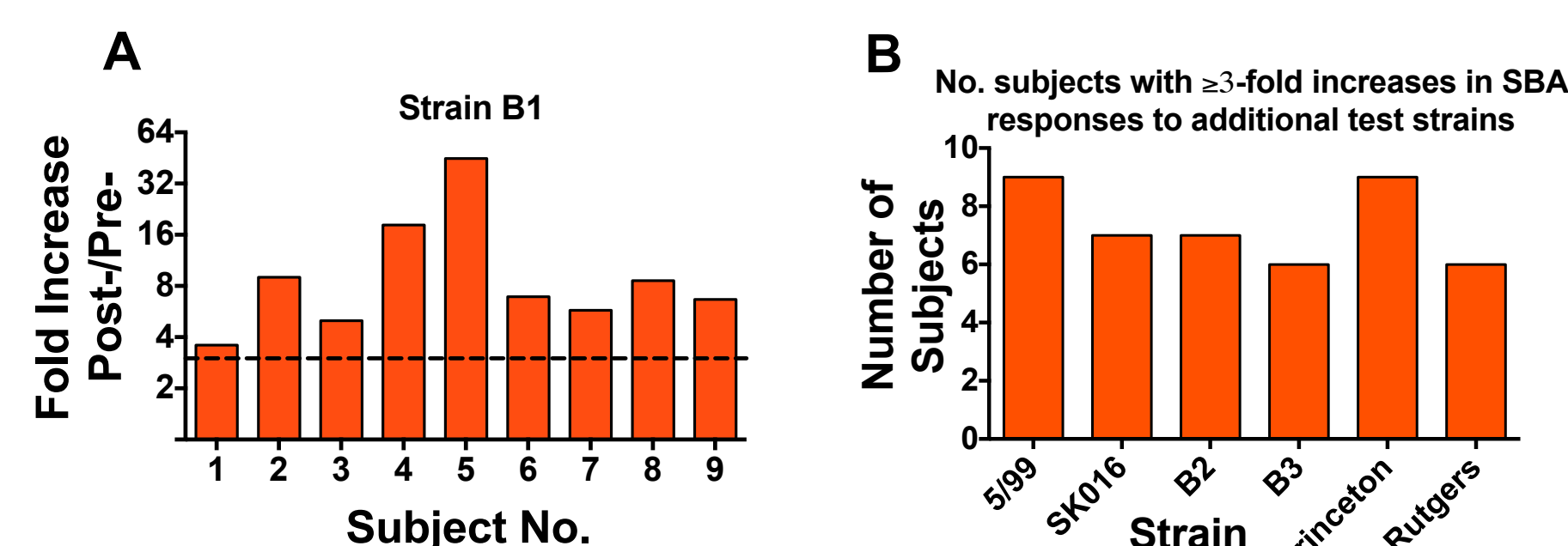


Figure 2. SBA responses of 9 subjects to strain B1 (Panel A), and number of subjects tested against 6 additional strains (Panel B)

Methods Continued

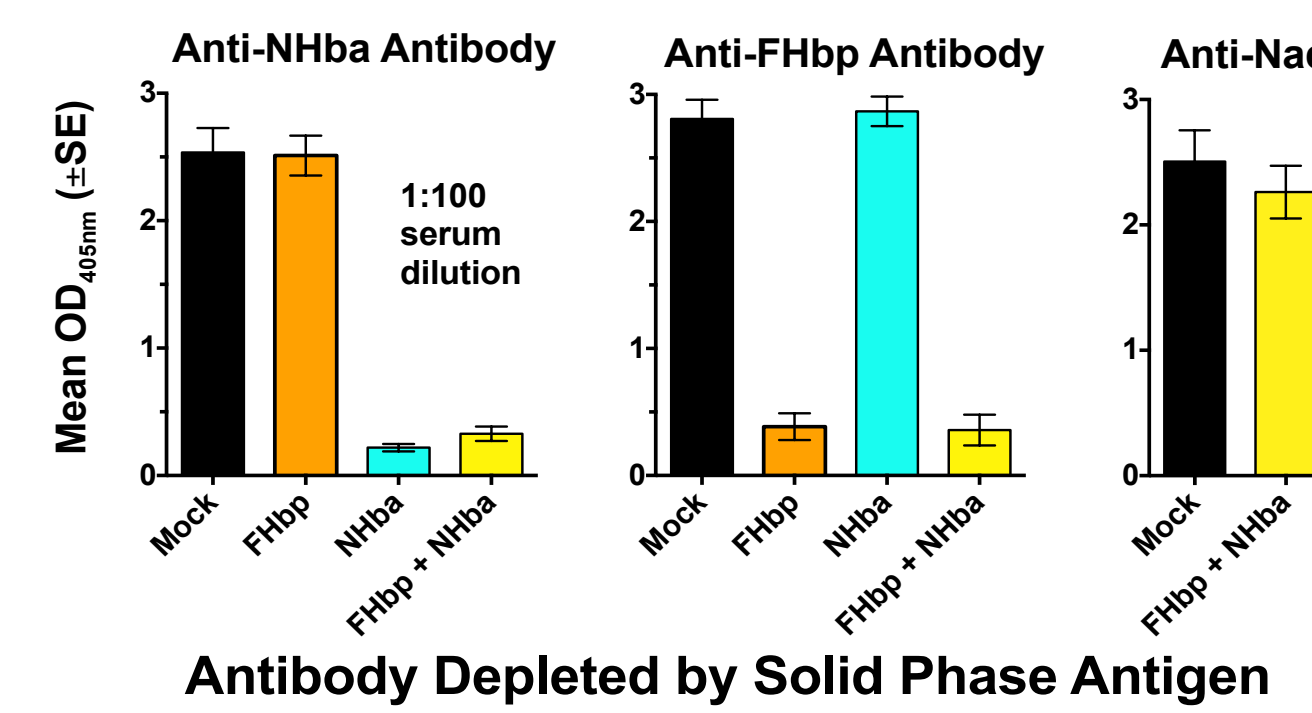
Strain	Antigen Matched with vaccine	FHbp Subfamily	NadA	NHba	PorA P 1.4
5/99	NadA	A	++	+/-	no
SK016	PorA P1.4	A	absent	+/-	yes
B1	NHba	A	absent	++ (100%)	no
B2	NHba	A	absent	++	no
B3	NHba	A	absent	+	no
Princeton	FHbp & NHba	B	absent	+(100%)	no
Rutgers	none	A	absent	+/-	no

Table 1. Meningococcal strain panel. Strains were considered matched with vaccine antigen by antibody binding to live bacteria via flow cytometry (FHbp, NadA, NHba) and by gene sequencing (FHbp subfamily B, PorA VR type 1.4 and presence of NadA gene).

Serum Antibody Depletion

- Sepharose columns were coupled to:
 - Recombinant FHbp and NHba
 - Bovine serum albumin (negative control, mock column)
- Serum was depleted of antibodies to FHbp, NHba, both FHbp + NHba, or mock
- By ELISA, 90% or more of the unwanted antibody was removed by adsorption with the specific antigen, without affecting titer of remaining antibodies (Figure 3)

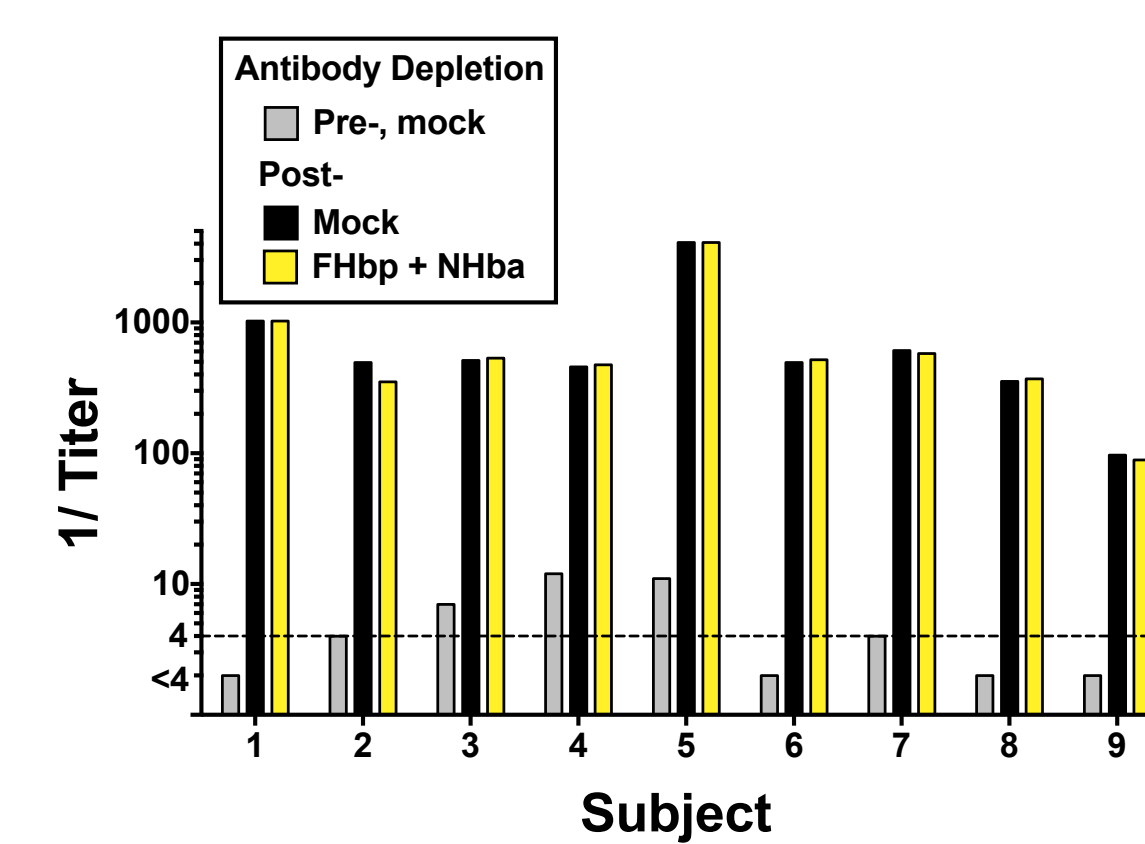
Figure 3. Adequacy of antibody depletion as measured by ELISA in post-immunization sera



Antibody Depleted by Solid Phase Antigen

Results

Figure 4. Effect of serum antibody depletion on SBA titers against control strain 5/99



Strain 5/99 is mismatched for all MenB-4C antigens except NadA. Depletion of both anti-FHbp and anti-NHba antibodies does not decrease SBA

Figure 5. Effect of serum antibody depletion on SBA titers against B1 strain

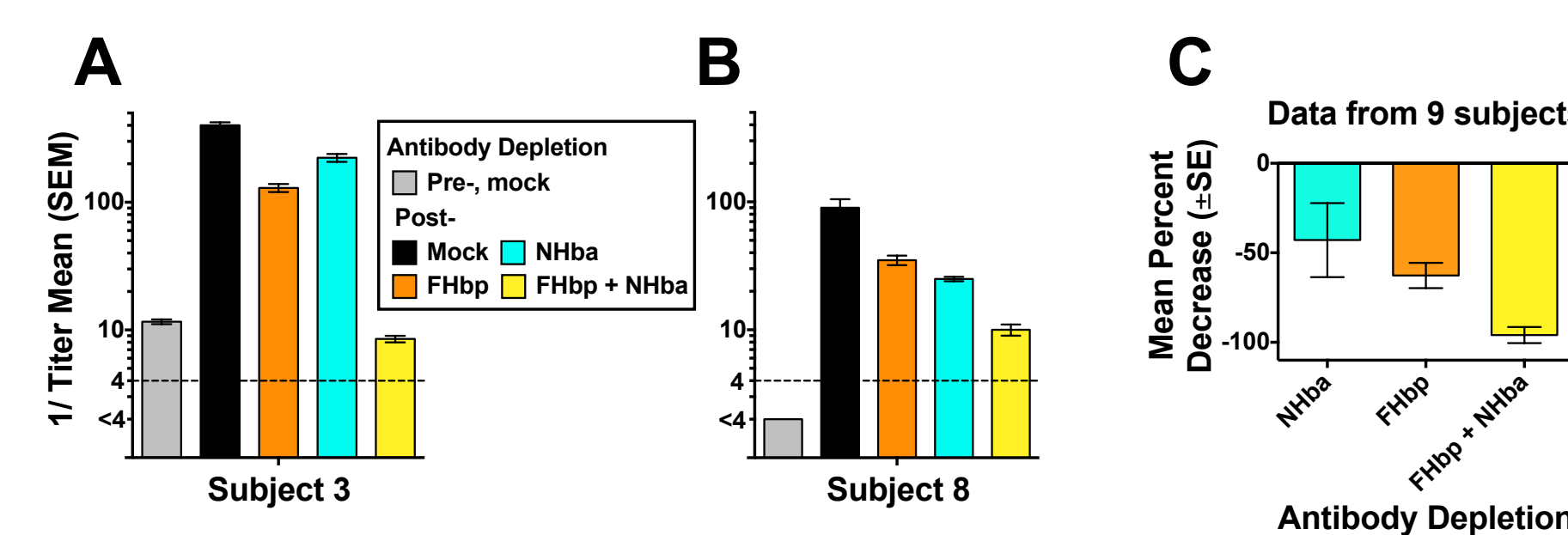


Figure 5. Strain B1. This strain expresses high NHba and is mismatched for the other 3 MenB-4C antigens. For Subject 3 (Panel A), depletion of either anti-NHba or anti-FHbp antibody removed only a small portion of the SBA but depletion of both antibodies removed all of the vaccine-induced SBA. Thus, either antibody can contribute to SBA. Similar results were seen for subject 8 (panel B), except that depletion of both antibodies did not return SBA titer to pre-vaccination level. Thus, vaccine-induced antibodies to other vaccine antigens (presumably OMV antigens, not PorA P1.4) can contribute to SBA. For all 9 subjects (panel C), depletion of anti-FHbp antibodies decreased SBA by a mean of 63% compared to 42% after depletion of anti-NHba antibodies. Depletion of both antibodies decreased SBA by a mean of 96%.

Figures 6 & 7. Effect of serum antibody depletion on SBA titers against Princeton Univ. and Rutgers Univ. strains

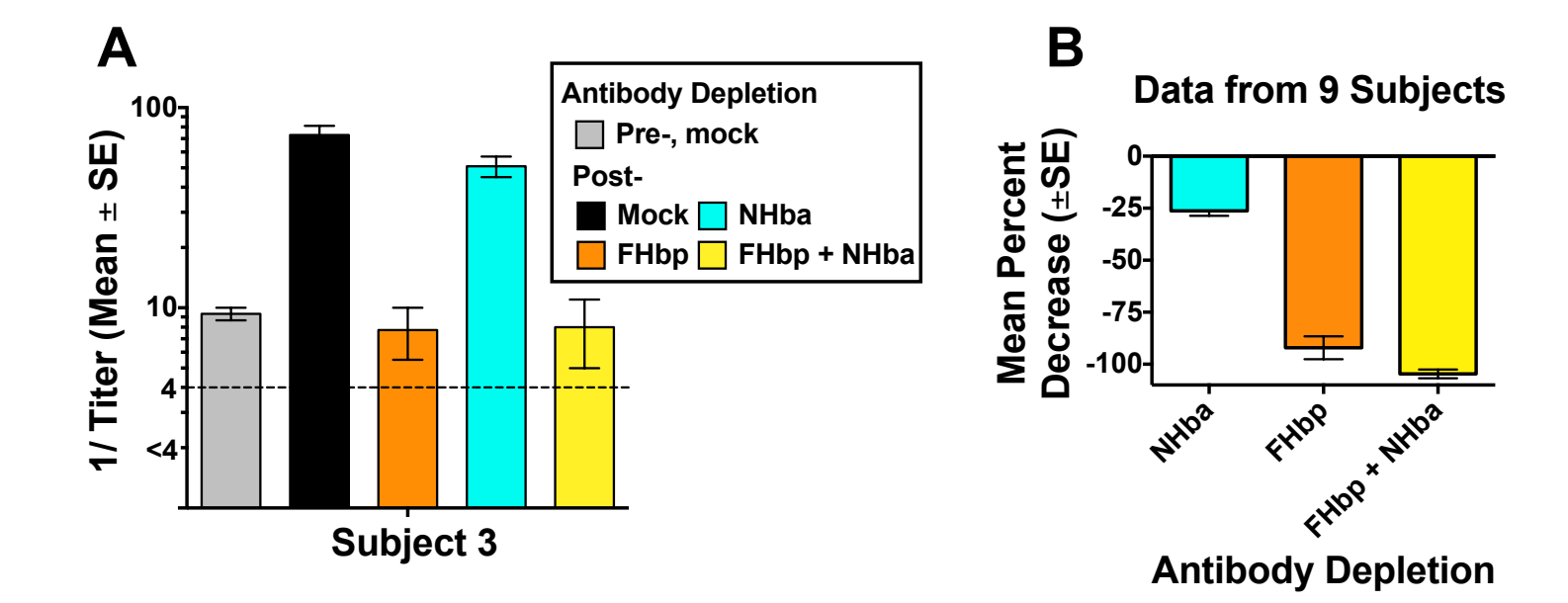


Figure 6. Princeton University strain. This strain is matched with MenB-4C for FHbp and NHba. For Subject 3 (panel A), depletion of anti-FHbp antibodies removed SBA titers to pre-vaccination level while depletion of anti-NHba antibodies had minimal effect. For all 9 subjects (Panel B), depletion of anti-FHbp antibody decreased SBA titers (mean=92%), whereas depletion of anti-NHba antibody had a smaller effect (mean =26%).

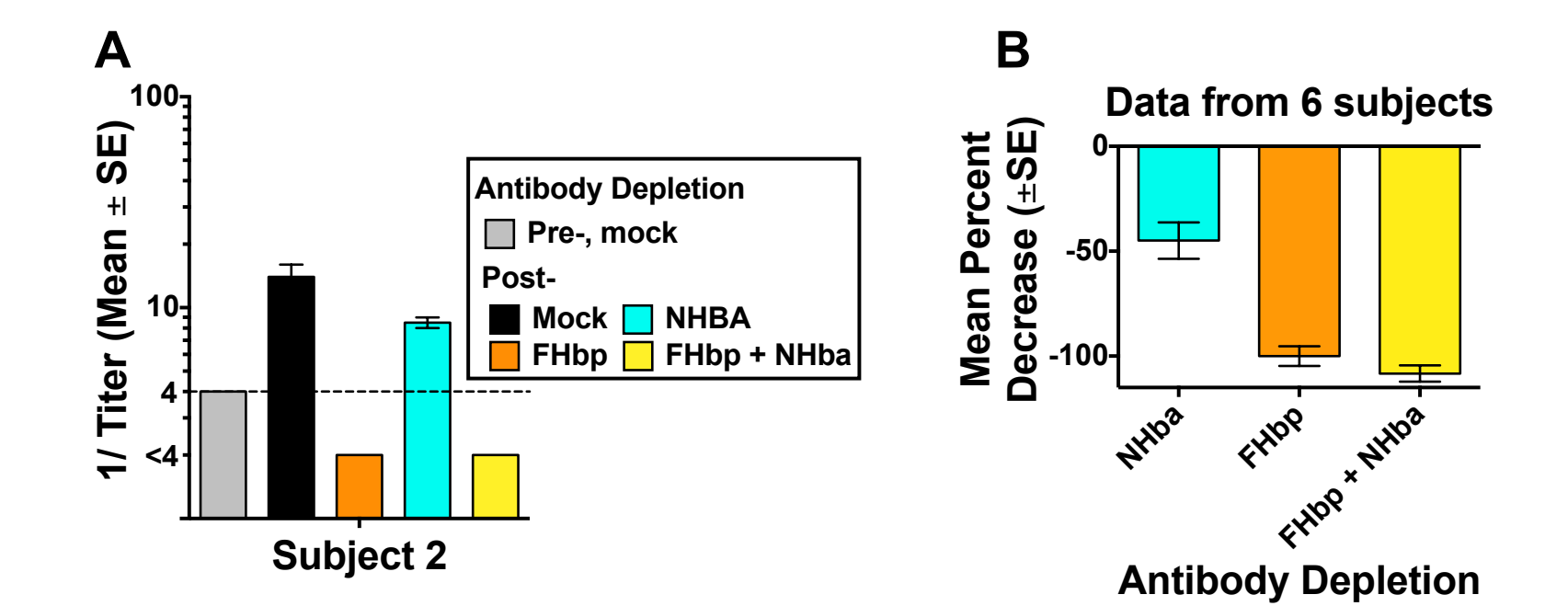


Figure 7. Rutgers University strain. This strain is mismatched for all 4 vaccine antigens. For Subject 2 (Panel A), depletion of anti-FHbp antibodies removed all of the SBA while depletion of anti-NHba antibodies had a minimal effect on SBA titer. For the 6 subjects tested against this strain (Panel B), depletion of anti-FHbp decreased SBA (mean of 100%) while anti-NHba depletion had a smaller effect (mean decrease of 45%).

Table 2. Antibody depleted sera that resulted in $\geq 50\%$ decrease in SBA against 5 test strains

Strain	No. of Post-immunization sera tested	No. of Sera (%) with $\geq 50\%$ decrease in SBA		Examples Shown
		Anti-NHba Depleted	Anti-FHbp Depleted	
B1	9	6 (67)	8 (89)	Figure 5
B2	6	6 (100)	2 (33)	Not shown
B3	7	3 (43)	4 (57)	Not shown
Princeton	9	0 (0)	9 (100)	Figure 6
Rutgers	6	3 (50)	6 (100)	Figure 7

Against three strains (B1, B2 and B3) matched with the vaccine only for NHba, antibodies to FHbp, NHba or both antibodies contributed to SBA depending on the subject. For the Princeton strain (matched with the vaccine for sub-family B FHbp and NHba), SBA depended nearly entirely on anti-FHbp antibody (the strain was resistant to anti-NHba). Against the Rutgers Univ strain, which is mismatched for all 4 vaccine antigens, depletion of anti-NHba antibodies decreased SBA in 3/6 sera tested while depletion of anti-FHbp antibodies decreased SBA in 6/6 sera.

Summary & Conclusions

- Some strains (such as B1) are susceptible to anti-NHba SBA, while others are resistant despite high expression of NHba with 100% amino acid identity to vaccine (i.e. Princeton strain)
- Occasionally strains are susceptible to anti-NHba SBA, mainly in concert with other vaccine-induced antibodies.
- Vaccine-induced serum antibodies to FHbp sub-family B can cross-react with strain FHbp sub-family A and elicit SBA in concert with antibodies to other antigens (i.e., NHba and/or OMV antigens that are not specific for PorA P1.4)
- The mechanisms by which MenB-4C elicit SBA are complex and vary depending on antibody composition of the serum, antigenic composition of the strain, and intrinsic strain factors that can affect complement regulation (such as binding of Factor H to NspA or PorB, Giuntini et al, Infect Immune 2015)