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MINIREVIEW

Does Binding of Complement Factor H to the Meningococcal Vaccine Antigen, Factor H Binding Protein, Decrease Protective Serum Antibody Responses?

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Factor H binding protein (fHbp) is a principal antigen in a multicomponent meningococcal vaccine recently licensed in Europe for prevention of serogroup B diseases. The protein recruits the complement downregulator, factor H (fH), to the bacterial surface, which enables the organism to resist complement-mediated bacteriolysis. Binding is specific for human fH. In preclinical studies, mice and rabbits immunized with fHbp vaccines developed serum bactericidal antibody responses, which in humans predict protection against developing meningococcal disease. These studies, however, were in animals whose fH did not bind to the vaccine antigen. Here we review the immunogenicity of fHbp vaccines in human fH transgenic mice. The data suggest that animals with high serum human fH concentrations have impaired protective antibody responses. Further, mutant fHbp vaccines with single amino acid substitutions that decrease fH binding are superior immunogens, possibly by unmasking epitopes in the fH binding site that are important for eliciting serum bactericidal antibody responses. Humans immunized with fHbp vaccines develop serum bactericidal antibody, but achieving broad coverage in infants required incorporation of additional antigens, including outer membrane vesicles, which increased rates of fever and local reactions at the injection site. The experimental results in transgenic mice predict that fHbp immunogenicity can be improved in humans by using mutant fHbp vaccines with decreased fH binding. These results have important public health implications for developing improved fHbp vaccines for control of serogroup B meningococcal disease and for development of vaccines against other microbes that bind host molecules.

VACCINE POTENTIAL OF MENINGOCOCCAL FACTOR H BINDING PROTEIN

Approximately one-third of cases of meningococcal disease in the United States (1), and an even higher proportion in Europe (2, 3), are caused by serogroup B strains. These strains are also responsible for a disproportionate number of cases in infants <1 year old (4) and can cause epidemics, such as the ones that occurred in New Zealand in the 1990s (5) and, more recently, in France (6). The serogroup B polysaccharide consists of α(2→8) *N*-acetylneuraminic acid, which is an auto-antigen (7). Use of this polysaccharide as a vaccine target therefore raised safety concerns. Alternative approaches for vaccine development against serogroup B strains used detergent-treated outer membrane vesicles (OMV) (8–10), native outer membrane vesicles (NOMV) from mutants with genetically attenuated endotoxin (11–13), or purified noncapsular antigens (reviewed in references 14–17). Specific examples of recombinant protein antigens include NspA (16), *Neisseria* heparin binding antigen (18) (also referred to as GNA2132 [19]), NadA (20), PorA (21), transferrin binding protein A (22), Opc outer membrane protein (23, 24), and factor H binding protein (fHbp; previously referred to as GNA1870 or LP 2086) (25, 26). One of the most promising protein antigens is fHbp, which is part of a multicomponent meningococcal vaccine recently licensed in Europe for immunization beginning at 2 months of age (27).

fHbp is a surface-exposed lipoprotein expressed by nearly all *Neisseria meningitidis* strains (28, 29). The protein recruits the complement downregulator, factor H (fH), to the bacterial surface (30), which enables the organism to evade innate immunity (30, 31). The vaccine antigen can be classified into two subfamilies (28) or three variant groups (25) based on cross-reactivity and amino acid sequence similarity. In infants and toddlers, antibodies to fHbp have complement-mediated bactericidal activity only against strains expressing an fHbp from the homologous subfamily or variant group closely matched to that of the vaccine antigen (32–34). In adolescents and adults, serum bactericidal antibody responses to fHbp vaccines appear to be broader than those in infants or toddlers (35, 36). In humans, serum bactericidal activity is the serologic hallmark of protection against developing meningococcal disease (37).

Anti-fHbp antibodies bind to the bacterial surface, activate the classical complement pathway directly, and block binding of fH (38). With less bound fH, the bacteria become more susceptible to anti-fHbp complement-mediated bacteriolysis because there is greater amplification of the alternative complement pathway (39). In many strains, fHbp is relatively sparsely exposed on the bacterial surface (38). Binding of anti-fHbp antibodies to these strains results in insufficient immune complex and, consequently, insufficient Fc density for efficient C1 complex engagement (38). As a result, complement activation via the classical pathway does not proceed to bacteriolysis in the absence of inhibition of fH binding and alternative pathway amplification (39, 40).

In 2009, we reported that binding of fH to fHbp was specific for human fH (41). Since preclinical fHbp immunogenicity studies

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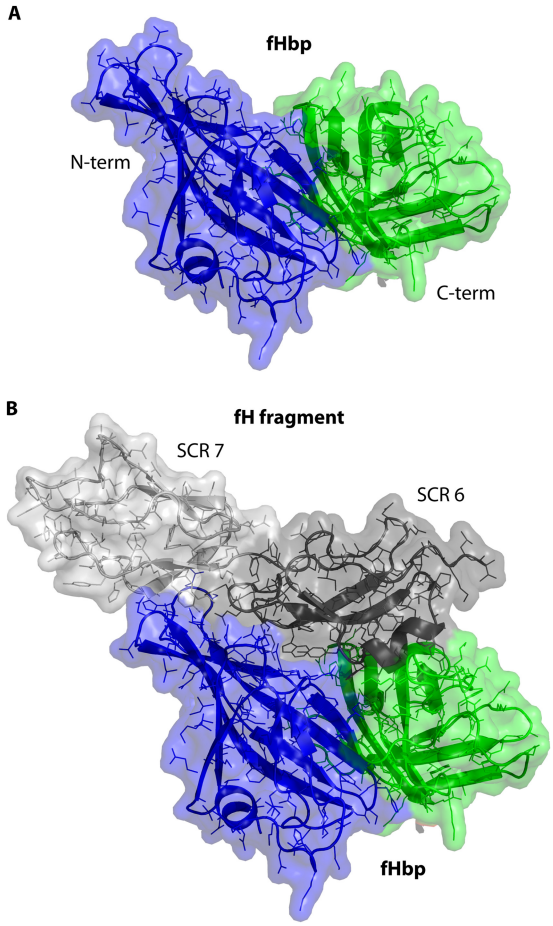

FIG 1 Structural models of fHbp. (A) Model of fHbp alone illustrating two domains, N-terminal (blue) and C-terminal (green). Mouse or rabbit fH does not bind to fHbp. In immunized mice or rabbits, epitopes in the fH binding site that are important for eliciting bactericidal antibody are exposed. (B) Model of fHbp in a complex with a fragment of fH (short consensus repeats [SCR] 6 and 7, gray). In humans, the vaccine antigen would be expected to be in a complex with fH and, possibly, mask epitopes in the fH binding site. Models are based on the coordinates of the published crystal structure (44).

had been done in mice and rabbits, the effect of binding of human fH to the vaccine on immunogenicity was not known. In previous studies, most mouse anti-fHbp monoclonal antibodies (MAbs) with bactericidal activity also inhibited binding of fH to fHbp, which suggested that the fHbp epitopes overlapped with the fH binding region in fHbp (42, 43). Conceivably, in immunized humans, fH forms a complex with this region of fHbp and masks important epitopes. A crystal structure of a fragment of fH in complex with fHbp subsequently provided a structural basis for the specificity of binding human fH (44) (Fig. 1).

The hypothesis that binding of a host molecule to a vaccine antigen impairs immunogenicity (45) can be tested by comparing vaccine immunogenicities in wild-type (WT) mice whose fH does not bind to the vaccine and human fH transgenic mice whose fH binds to the vaccine (46–48). Further evidence is provided by comparative immunogenicity studies in transgenic mice testing mutant fHbp antigens engineered to have decreased fH binding compared with the corresponding antigen that binds fH (46–48).

The purpose of this article is to review data that address whether binding of human fH to fHbp impairs fHbp vaccine immunogenicity and whether mutant fHbp vaccines with decreased fH binding can overcome this impairment. Two published studies reported that mutant fHbp vaccines consisting of either a recombinant protein or native outer membrane vesicles elicited superior serum bactericidal antibody responses in human fH transgenic mice compared with the respective control fHbp vaccines that strongly bind fH (46, 47). A third recently published study suggested that binding of human fH to fHbp did not attenuate immunogenicity (48). In this review, we highlight the differences in methodology and interpretations of these studies to understand better the seemingly disparate observations. As noted above, recombinant fHbp antigens that bind fH are part of vaccines licensed or being developed for prevention of meningococcal disease (33, 34, 49). If fH binding impairs immunogenicity, the effectiveness of these fHbp vaccines in humans can be improved by introduction of amino acid substitutions that decrease or eliminate binding of fH (47, 48, 50). Understanding the effects of these substitutions, therefore, has important scientific, clinical, and public health relevance to the design of optimally immunogenic meningococcal fHbp vaccines.

HUMAN fH TRANSGENIC MICE HAVE LOWER SERUM BACTERICIDAL ANTIBODY RESPONSES TO fHbp VACCINES THAT BIND HUMAN fH THAN DO WILD-TYPE MICE WHOSE fH DOES NOT BIND TO THE VACCINE

To investigate the effect of fH binding on fHbp immunogenicity, we used a human fH transgenic mouse model that was developed by Peter Rice and colleagues at the University of Massachusetts Medical School (47). These mice were generated by microinjecting BALB/c mouse embryos with a recombinant DNA molecule containing a cytomegalovirus (CMV) enhancer, a chicken β-actin promoter, cDNA encoding the full-length human fH protein, and a rabbit β-globin poly(A) sequence. Sera from mice containing the transgene expressed the full-length human fH protein by Western blotting (Fig. 2A). The CMV enhancer and chicken β-actin promoter were used to achieve high levels of human fH expression to approximate the serum fH concentrations of humans. Using an fHbp capture enzyme-linked immunosorbent assay (ELISA) (47), the median concentration of human fH in the mouse sera was 235 μg/ml, which was similar to that of control human sera assayed in parallel (291 μg/ml; $P = 0.22$, Mann-Whitney test) (Fig. 2B). However, the range of human fH concentrations in the transgenic mouse sera was greater than the range of fH levels in human sera (P value of 0.008 for differences in the respective variances). Note that in previous studies, the mean fH concentrations in normal human sera or plasma ranged from 210 to 516 μg/ml (51–54), a range which was consistent with the concentrations in the control human sera measured by the fHbp capture ELISA.

The BALB/c transgenic mice expressed both mouse and human fH (Table 1). The mice appeared to be generally healthy and, when immunized with a control meningococcal serogroup C polysaccharide-protein conjugate vaccine, had serum anti-capsular and anti-carrier protein IgG antibody responses and bactericidal antibody responses similar to those of control BALB/c mice whose sera were negative for human fH (47). In contrast, when the transgenic mice were immunized with an fHbp vaccine that bound fH, the geometric mean serum bactericidal titers in two replicate studies were 4- to 8-fold lower than those of the control

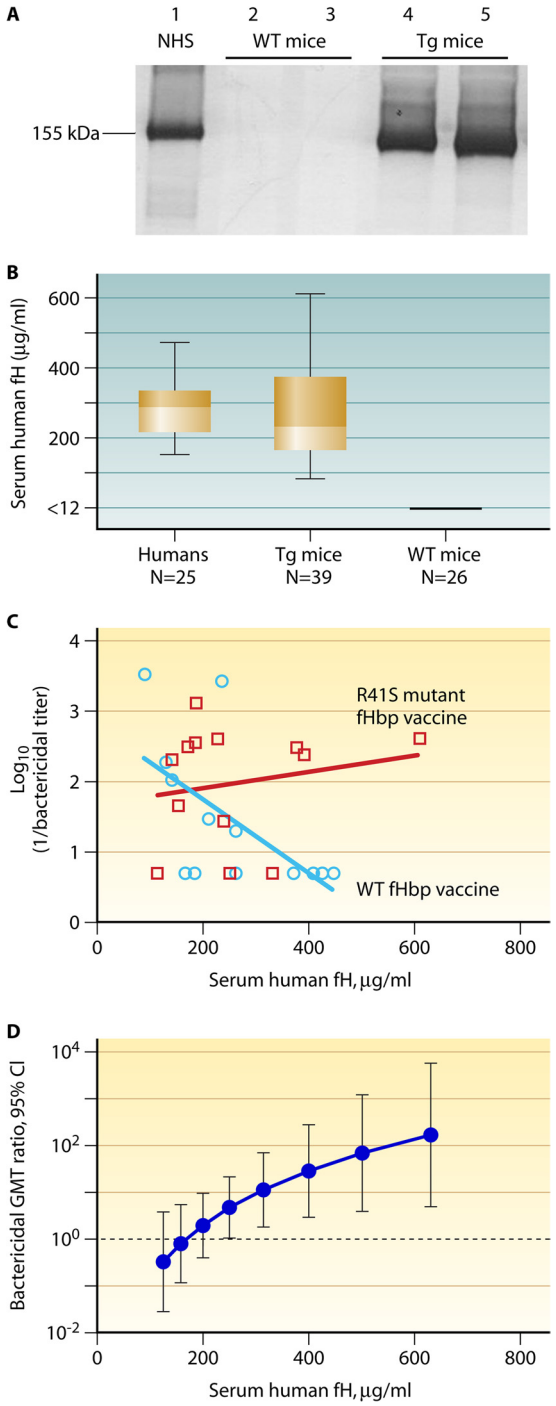

FIG 2 Immunogenicity of mutant and WT fHbp vaccines in human fH transgenic mice in relation to serum human fH concentrations. (A) Detection of human factor H in sera from fH transgenic (Tg) mice by Western blotting. Lane 1, normal human serum (NHS; diluted 1:100); lanes 2 and 3, individual normal (BALB/c) mouse sera (diluted 1:25); lanes 4 and 5, individual transgenic mouse sera (diluted 1:25). Membrane was probed with affinity-purified goat anti-human fH (Complement Technology Inc.) that recognized human, but not mouse, fH. The image is from a replicate experiment, which was performed as part of a previous study (47). (B) Concentrations of human fH in mouse sera measured by a capture ELISA. Positive mice had human fH concentrations of >90 μg/ml, and negative mice had concentrations of <12 μg/ml, which was the lower limit of detection in the assay. For comparison, concentrations of human fH were measured in parallel in stored sera from 25 healthy adult humans. The boxes in the graph extend from the 25th to 75th percentiles. The lines in the middle represent the median values. The whiskers extend from the lowest to the highest value. Data points used to calculate the plots are from Beernink et al. (47). (C) Relationship between serum bactericidal antibody titers measured against serogroup B strain H44/76 and serum human fH concentrations of transgenic mice. The blue circles represent the bactericidal antibody responses of mice immunized with the wild-type control fHbp vaccine that strongly bound fH, measured against serogroup B strain H44/76. The inverse correlation with serum fH concentrations was significant ($r = -0.65$; $P = 0.02$; Pearson correlation coefficient between $\log_{10}$ [1/bactericidal titer] and $\log_{10}$ [fH concentration]). The red squares represent the corresponding bactericidal responses of transgenic mice immunized with the R41S mutant fHbp vaccine. The correlation with the serum fH concentrations was not significant ($r = +0.18$; $P = 0.58$). The respective r values for the two vaccines were significantly different ($P = 0.03$). The plots were calculated from previously published data of Beernink et al. (47). (D) Effect of serum human fH concentrations on the ratio of bactericidal antibody responses of transgenic mice immunized with an R41S mutant fHbp vaccine to those of mice immunized with a control fHbp vaccine that strongly bound human fH. The ratios of the geometric mean bactericidal responses of the group immunized with R41S fHbp vaccine to those of the group immunized with control fHbp vaccine were significantly greater than 1 (in favor of the mutant fHbp vaccine) for all human fH concentrations of >250 μg/ml by general linear regression ($P < 0.05$) and for human fH concentrations of >316 μg/ml ($P < 0.01$) (47). (Originally published in P. T. Beernink, J. Shaughnessy, E. M. Braga, Q. Liu, P. A. Rice, S. Ram, and D. M. Granoff. 2011. A meningococcal factor H binding protein mutant that eliminates factor H binding enhances protective antibody responses to vaccination. J. Immunol. 186:3606–3614. Copyright © 2011 The American Association of Immunologists, Inc.)

mice whose sera were negative for human fH (study 1, $P = 0.03$; study 2, $P = 0.05$) (47). Further, in the transgenic mice, there was an inverse correlation between the serum fH levels and the magnitudes of the vaccine response: the higher the serum human fH concentrations, the lower were the serum bactericidal titers to the fHbp vaccine that bound human fH (Pearson correlation coefficient, $r = -0.65$; $P = 0.02$) (Fig. 2C, blue line).

In a subsequent study, we immunized human fH transgenic and wild-type mice with a NOMV vaccine prepared from a mutant meningococcal strain with attenuated endotoxin (LpxL1 knockout) and overexpressed fHbp that bound fH (46). In the normal mice whose fH did not bind to the fHbp vaccine, two doses of the NOMV vaccine with overexpressed WT fHbp elicited higher serum bactericidal anti-fHbp antibody responses than did three doses of the same vaccine given to the human fH transgenic mice whose human fH bound to the vaccine (compare Fig. 3A and B). Thus, in two studies with different fHbp vaccines that strongly bound fH, the human fH transgenic mice had lower serum bactericidal responses than normal mice whose mouse fH did not bind to the vaccines. In contrast, as noted above, the transgenic and normal mice had indistinguishable serum bactericidal antibody responses to a control meningococcal serogroup C conjugate vaccine (47). Therefore, the impairment of the responses of the transgenic mice was specific for the fHbp vaccine that bound human fH. These data supported the hypothesis that binding of a host molecule to a vaccine antigen impaired immunogenicity.

MUTANT fHbp VACCINES WITH DECREASED fH BINDING ARE SUPERIOR IMMUNOGENS IN HUMAN fH TRANSGENIC MICE WITH HIGH SERUM HUMAN fH CONCENTRATIONS

We also immunized human fH transgenic mice with a mutant fHbp vaccine containing a single amino substitution (Arg at residue 41 was replaced by Ser; R41S), which resulted in substantially decreased binding of fH (Fig. 4) (47). In human transgenic mice,

TABLE 1 Comparison of the methods used to investigate mutant fHbp vaccines in studies using transgenic mice

Parameter	Value(s) for each mutant fHbp vaccine (reference)[a]		
	Recombinant fHbp (47)	NOMV (46)	Recombinant fHbp (48)
Vaccine dose (μg of protein)	20	2.5	20
Antigen	fHbp R41S-His_6	NOMV expressing fHbp R41S	fHbp E218A/E239A, R41A, and I246A (each with His_6)
Adjuvant	Aluminum hydroxide	Aluminum hydroxide	Aluminum hydroxide
No. of doses of vaccine (route)	3 (i.p.)	3 (i.p.)	3 (i.p.)
Bactericidal group B target strain	H44/76 (fHbp ID 1; PorA VR type 7,16)	Cu385 (fHbp ID 1; PorA VR type 19,15)	MC58 (fHbp ID 1; PorA VR type 7,16-2)
Mouse genetic background	BALB/c	BALB/c	C57BL/6
Age at immunization (mo)	1.5 to 2	2 to 4	3 to 4
Sex	Males and females	Males and females	Not reported
Mouse fH	Present	Present	Absent
Transgenic fH	Full-length human fH	Full-length human fH	Chimeric mouse-human fH[b]
Enhancer/promoter	CMV/chicken β-actin	CMV/chicken β-actin	None/*apoE*
Serum human or chimeric fH (mean [range]) (μg/ml)	268 (89 to 610)	427[c] (249 to 788)	Not reported[d]

[a] All three studies used WT fHbp ID 1 in variant group 1 for preparing the mutant and control fHbp vaccines. The amino acid residues in the mutants are numbered based on the mature fHbp ID 1 protein sequence (http://pubmlst.org/neisseria/fHbp). i.p., intraperitoneal.
[b] The chimeric fH molecule consisted of human short consensus repeat domains (SCRs) 6 to 8 flanked by the mouse sequences for SCRs 1 to 5 and SCRs 9 to 20.
[c] Only mice that expressed levels of human fH of >240 μg/ml were selected for use in this study.
[d] The serum concentrations of the chimeric fH in the transgenic mouse line were reported in a previous publication to be between 92 and 210 μg/ml (55).

the fHbp mutant with decreased fH binding elicited antibodies with greater bactericidal activity than the control fHbp vaccine that bound fH. Overall, the increase in the geometric mean titer (GMT) was only 3-fold. However, in contrast to the results with the fHbp vaccine that bound fH, with the mutant vaccine that did not bind human fH, there was no significant correlation between the serum human fH concentrations and bactericidal responses (Pearson correlation coefficient, $r = 0.18$; $P = 0.57$) (Fig. 2C, red line). The test of equality of the two Pearson correlation coefficients showed that the respective correlations between the vaccine groups were significantly different from each other ($P = 0.03$). Thus, the reason for the relatively small overall increase in the titers elicited by the mutant vaccine likely was the lack of impairment of serum bactericidal antibody responses to the control fHbp vaccine that bound fH when the serum human fH concentrations of the transgenic mice were low (Fig. 2C). Indeed, using general linear regression, we found a statistically significant effect on enhanced immunogenicity of the fHbp, with decreased fH binding only in transgenic mice with serum concentrations of human fH of ≥250 μg/ml ($P < 0.05$) (Fig. 2D). The model indicated ~5-fold enhanced immunogenicity of the mutant fHbp vaccine compared with that of the vaccine that bound fH when the serum human fH concentration was ~250 μg/ml and ~28-fold enhanced immunogenicity when the serum human fH concentration was ~400 μg/ml ($P < 0.01$). We also found that the anti-fHbp antibodies elicited by the mutant fHbp vaccine had greater inhibitory activity for fH binding to fHbp than anti-fHbp antibodies elicited by the control fHbp vaccine that strongly bound fH (47). Thus, unmasking epitopes in the fH binding site of the mutant vaccines appears to affect the anti-fHbp antibody repertoire, and the higher bactericidal activity elicited by the mutant vaccines may have resulted from antibodies with greater fH inhibition, which resulted in less fH bound to the bacteria.

In the second study with NOMV vaccines, we immunized human fH transgenic mice with a NOMV vaccine with genetically attenuated endotoxin activity and overexpressed WT fHbp, which bound fH strongly, or overexpressed R41S mutant fHbp, which had decreased fH binding (46). The two vaccines had similar levels of fHbp expression. The transgenic mice immunized with the NOMV vaccine with the R41S mutant fHbp had a 19-fold-higher serum bactericidal GMT than the transgenic mice immunized with the control NOMV vaccine with WT fHbp that bound human fH (Fig. 3A). The test strain had a PorA heterologous to that of the vaccine (Table 1), and by antibody depletion studies, the serum bactericidal activity was shown to be directed against fHbp (46). In this second study, we immunized transgenic mice with only serum human fH concentrations of 250 μg/ml or greater. This selection criterion likely contributed to the larger overall increase in immunogenicity of the NOMV vaccine containing the mutant fHbp that did not bind human fH compared to that observed in our first study with the recombinant mutant fHbp vaccine (47).

Our hypothesis is that the enhanced immunogenicity in human fH transgenic mice of mutant fHbp vaccines with decreased fH binding was not a result of increased immunogenicity of the mutant fHbp vaccines *per se* but from decreased immunogenicity of the fHbp vaccines that bound human fH when serum human fH concentrations were high. Note that in normal BALB/c mice in which mouse fH did not bind to the control or mutant fHbp vaccines, the NOMV-fHbp vaccine with the R41S mutation had decreased serum bactericidal antibody responses compared to those of the control NOMV-fHbp vaccine without the mutation ($P = 0.003$) (Fig. 3B). Thus, in the absence of human fH, introduction of the R41S amino acid substitution decreased vaccine immunogenicity. This moderate loss of immunogenicity of the mutant fHbp vaccine, which was evident only in normal mice, was more than compensated for in human fH transgenic mice by the greater effect of human fH on decreasing immunogenicity of the NOMV vaccine containing the control fHbp that bound human fH.

Based on protein phylogeny, fHbp variants can be subclassified

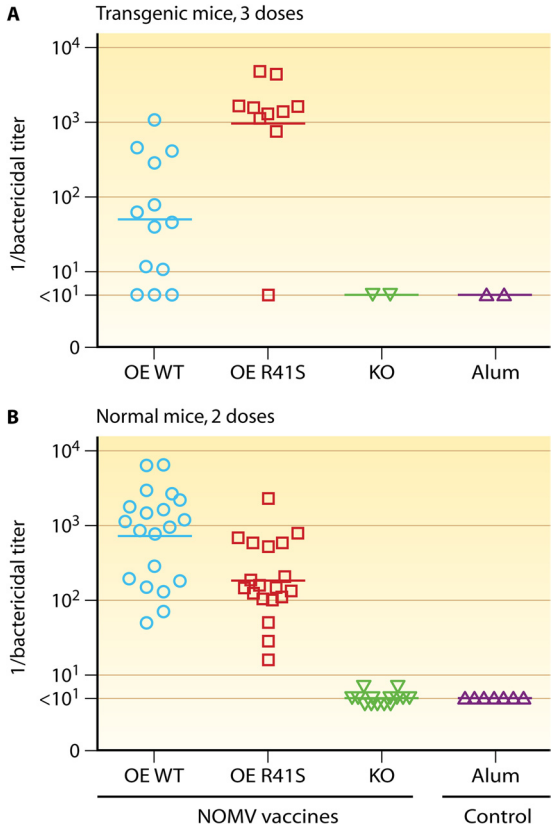

FIG 3 Serum bactericidal antibody responses of human fH transgenic mice immunized with NOMV vaccines with overexpressed fHbp. Each symbol represents the reciprocal serum titer of an individual mouse; the horizontal lines represent the geometric mean titers. The NOMV vaccines were prepared from mutants of group B strain H44/76 with genetically attenuated endotoxin (LpxL1 knockout) and overexpressed (OE) WT or R41S mutant fHbp ID 1. The test strain, Cu385, has a mismatched PorA variable region type compared to the vaccine strain and expressed fHbp ID 1 that matched the vaccine fHbp antigen (Table 1). OE WT, NOMV vaccine with overexpressed wild-type (WT) fHbp that bound human fH; OE R41S, NOMV vaccine with overexpressed mutant R41S fHbp with lower binding to human fH; KO, control NOMV vaccine from the fHbp knockout; Alum, aluminum hydroxide adjuvant only. (A) Human fH transgenic mice immunized with three injections of vaccine. All mice had serum human fH concentrations of ≥250 μg/ml. The NOMV vaccine with the mutant fHbp elicited 19-fold-higher titers than the NOMV vaccine with WT fHbp that strongly bound human fH ($P = 0.001$). (B) Normal BALB/c mice whose mouse fH does not bind to fHbp were immunized with two doses of vaccine. The NOMV vaccine with WT fHbp elicited 4-fold-higher titers than the NOMV vaccine with the mutant fHbp ($P = 0.003$). (Reprinted from reference 46 with permission of the publisher.)

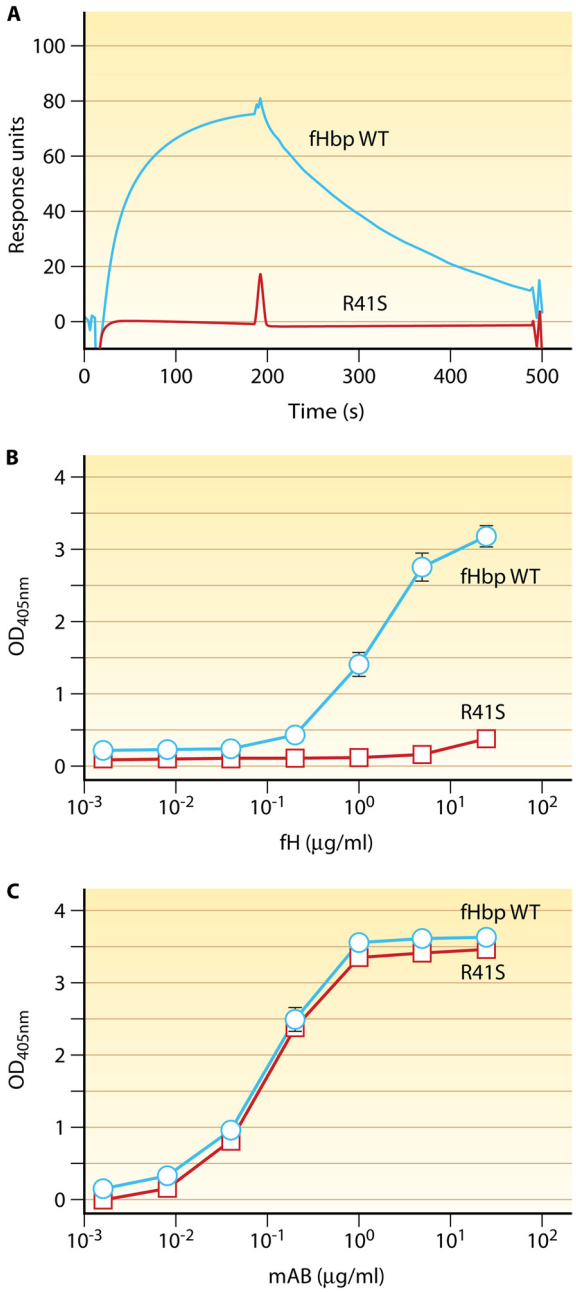

FIG 4 Replacement of arginine at residue 41 by serine (R41S) decreases binding of fH. (A) By surface plasmon resonance, the fHbp WT (blue line) bound immobilized human fH whereas the R41S mutant (red line) had no detectable binding to fH. Data are for 62.5 nM injection of each fHbp analyte. (B) By ELISA, the fHbp WT (circles with blue line) bound fH, whereas with the R41S substitution (squares with red line), there was no detectable binding of soluble human fH to solid-phase mutant fHbp. OD_{450}, optical density at 450 nm. (C) Binding of anti-fHbp MAb JAR4 to solid-phase wild-type or R41S mutant fHbp indicated that similar amounts of the two proteins were adsorbed to the wells of the microtiter plate and that a conformational epitope in the N-terminal domain was retained by the mutant. (Originally published in P. T. Beernink, J. Shaughnessy, E. M. Braga, Q. Liu, P. A. Rice, S. Ram, and D. M. Granoff. 2011. A meningococcal factor H binding protein mutant that eliminates factor H binding enhances protective antibody responses to vaccination. J. Immunol. 186:3606–3614. Copyright © 2011 The American Association of Immunologists, Inc.)

into three variant groups (25). Interestingly, the R41S substitution, which eliminated fH binding to fHbp in variant group 1, did not affect fH binding to fHbps in variant group 2 (50). In this study, we identified several other amino substitutions in fHbp in variant group 2 that greatly decreased binding of fH (50). Recently, we investigated the immunogenicity of two of these mutant recombinant fHbp vaccines in human fH transgenic mice. Again, we excluded mice with serum human fH concentrations of <250 μg/ml and observed significantly higher serum bactericidal antibody responses to the mutant fHbp vaccines with low binding to human fH than to the control fHbp vaccine that bound fH ($P \leq 0.001$) (D. M. Granoff and P. T. Beernink, unpublished data). Thus, in three studies in human fH transgenic mice, we observed

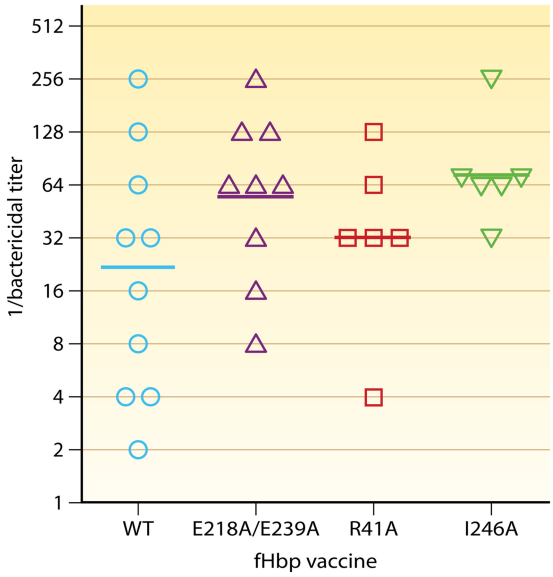

FIG 5 Serum bactericidal antibody responses of transgenic mice immunized with mutant recombinant fHbp vaccines that do not bind fH. C57BL/6 mice that lacked endogenous mouse fH and expressed a chimeric mouse-human fH molecule that permitted binding to fHbp were immunized with three doses of a wild-type (WT) fHbp vaccine that bound fH or three mutant vaccines with decreased fH binding (E218A/E239A double mutant, R41A, or I246A). The serum bactericidal GMT of mice given the WT fHbp vaccine was 1:22, which was lower than that for each of the three mutant fHbp vaccines tested (1:55, 1:32, and 1:72); none of the pairwise comparisons with the WT vaccine were significant ($P > 0.05$). Geometric mean titers (GMT) are shown as horizontal bars as calculated from data in Fig. 5B of the original paper (48). The amino acid residues have been renumbered based on the mature fHbp ID 1 protein sequence (http://pubmlst.org/neisseria/fHbp). (Modified from reference 48 with permission of the publisher.)

higher serum bactericidal antibody responses to mutant fHbp vaccines designed to have decreased fH binding than titers elicited by the respective control fHbp vaccines that strongly bound human fH. In contrast, a recently published study by Johnson and colleagues cast doubt about the superior immunogenicity in transgenic mice of mutant fHbp vaccines with lower fH binding (48). Below we discuss some possible reasons for the discordant results.

Johnson et al. (48) immunized human fH transgenic mice with three different mutant fHbp vaccines with decreased binding of fH (Fig. 5). The authors reported no significant differences between the serum bactericidal antibody responses of transgenic mice immunized with mutant fHbp vaccines with decreased fH binding and those immunized with a control wild-type fHbp vaccine that strongly bound fH (Fig. 5). The serum bactericidal GMT of mice given the control fHbp vaccine was 1:22, which was lower than that for each of the three fHbp vaccines tested with decreased binding of human fH (1:55, 1:32, and 1:72; GMTs calculated from their data in Fig. 5B of the original paper [48]). Since none of the pairwise comparisons with the control fHbp vaccine that bound fH was statistically significant, the authors concluded that there was no enhancement of immunogenicity by the mutant fHbp vaccines that did not bind human fH.

Johnson et al. (48) used a previously described transgenic mouse model that was generated in a C57BL/6 background (55) (Table 1). These transgenic mice lacked endogenous mouse fH and expressed a chimeric mouse-human fH molecule that bound to fHbp. Since the short consensus repeat (SCR) domains 6 and 7 of human fH are known to interact with fHbp (44), Johnson et al. employed a chimeric fH molecule that consisted of human SCR domains 6 to 8 flanked by the mouse sequences for SCR domains 1 to 5 and 9 to 20. While the authors proposed that this transgenic model provided a physiologically relevant model to investigate the immunogenicity of WT and mutant fHbp vaccines with decreased binding of human fH, they did not quantify the fH concentrations in the mouse sera. This transgenic mouse line in which fH expression was driven by the *apoE* promoter (Table 1) originally was reported by Ufret-Vincenty et al. to have serum concentrations of chimeric fH ranging between 92 and 210 μg/ml (55). The published mean serum fH concentrations in humans lie between 210 and 516 μg/ml (47, 51–54). Given our previous report that the superior immunogenicity of mutant fHbp vaccines with low fH binding was observed only in transgenic mice with serum human fH levels of ≥250 μg/ml (Fig. 2D), it is not surprising that Johnson et al. did not observe statistically significant differences in immunogenicity between the control and mutant fHbp vaccines tested in their transgenic mice (48).

Johnson et al. also did not observe a relationship between serum chimeric fH concentrations and serum bactericidal titers in individual transgenic mice (see supplemental Fig. 4 of their publication [48]). The data reported, however, were from transgenic mice immunized with mutant fHbp vaccines in which a lack of correlation was expected, since, in addition to low serum chimeric fH concentrations, the vaccines tested did not bind fH. We, too, did not find a correlation between serum human fH concentrations and serum bactericidal antibody responses of human fH transgenic mice immunized with the R41S mutant fHbp vaccine (Fig. 2C, red regression line). Indeed, these results added credence to the significant inverse correlation we found in transgenic mice immunized with the fHbp vaccine that bound human fH. Thus, in our studies, the impaired serum bactericidal antibody responses of the transgenic mice were specific for the fHbp vaccine that bound human fH when the human fH concentrations were sufficiently high.

We acknowledge that a direct causal link between high serum human fH concentrations and decreased fHbp immunogenicity has not been demonstrated. Apart from the differences in human or chimeric fH concentrations in the two transgenic mouse models, we also cannot exclude the possibility that other differences in study design or reagents might have contributed to the different results reported by Johnson et al. (48) and our group. Most notably, these include different genetic backgrounds of the transgenic mouse strains, use of a mouse line expressing both intact mouse and human fH versus another expressing only chimeric mouse-human factor H, and slight differences in the immunization protocols (Table 1) or the bactericidal assay methods. In addition, potential differences in the affinities of full-length human fH and the chimeric fH for the control fHbp vaccine may possibly have contributed to the differences in immunogenicity. While human fH binds WT fHbp with affinities in the mid-nanomolar range (56, 57), the affinity of the chimeric fH for WT fHbp is not known.

OVERALL CONCLUSIONS

In two published studies, and in a third study not yet published (Granoff and Beernink, unpublished data), we found superior bactericidal antibody responses elicited by mutant fHbp vaccines

compared to the respective serum bactericidal responses to control fHbp vaccines that strongly bound human fH. Collectively, the results provide strong evidence that human fH decreases fHbp immunogenicity and that high serum fH concentrations correlate with decreased fHbp immunogenicity. Had we only analyzed the antibody responses of the transgenic mice with serum human fH levels of <250 μg/ml (47), we, too, would not have observed significant differences in immunogenicity between the control and mutant recombinant fHbp vaccines. Thus, the most likely explanation for the lack of superior immunogenicity of the mutant fHbp vaccines tested by Johnson et al. (48) was their use of a transgenic mouse model with serum chimeric fH concentrations that were lower than those expressed in our transgenic model and lower than those found in many healthy humans.

The fHbp antigen in the recently licensed meningococcal serogroup B vaccine is a fusion protein with GNA 2091 (49). The ID 1 fHbp sequence variant used in the fusion protein is known to be a high binder of human fH (56). In human infants and toddlers, the breadth of coverage elicited by the recombinant protein antigens when administered without the OMV component in the multicomponent vaccine (referred to as 3MenB vaccine) was limited (32, 33). One explanation for the limited coverage is an effect of human fH on decreasing fHbp immunogenicity. The addition of OMV (referred to as 4MenB vaccine) improved vaccine coverage (32, 33). In the Findlow et al. study, however, the addition of the OMV was accompanied by higher rates of fever and greater inflammatory reactions at the injection sites than when the recombinant proteins were given without OMV (32). In a subsequent larger study, fever of 39°C or higher occurred in 10% to 15% of infants given the 4MenB vaccine along with routinely recommended vaccines, compared with 3% to 4% of control infants given recommended vaccines only (58). While the clinical importance of these reactions will need to be defined by postlicensure studies in large populations (59), the potential of improving fHbp immunogenicity by using mutant molecules with decreased fH binding may improve vaccine coverage and, possibly, avoid inclusion of the OMV vaccine.

The bivalent fHbp vaccine under development (34) contains recombinant fHbp sequence variants ID 55 (also referred to as B01) in variant group 1 and ID 45 (also referred to as A05) in variant group 3 (34). These two proteins avidly bind human fH (1.6- and 5.2-fold-higher binding than fHbp ID 1, respectively) (56). In toddlers, the bivalent vaccine elicited a limited breadth of bactericidal antibody against four of six primary test strains with fHbp in variant group 1, 2, or 3 (34). Conceivably, using mutant fHbp molecules with decreased fH binding might also improve the breadth of coverage of this vaccine. The prospect of improving vaccine immunogenicity against a potentially fatal disease has important implications for global public health and merits further study, including testing immunogenicity of mutant fHbp vaccines in humans. Indeed, while Johnson et al. did not observe enhanced immunogenicity of mutant fHbp molecules with decreased fH binding, they also concluded that clinical trials were needed to provide definitive evidence of whether the mutants offered superior safety and immunogenicity compared with those of wild-type fHbp vaccines that bind human fH (48).

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