A Meningococcal Factor H Binding Protein Mutant That Eliminates Factor H Binding Enhances Protective Antibody Responses to Vaccination

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*J Immunol* published online 16 February 2011
http://www.jimmunol.org/content/early/2011/02/14/jimmunol.1003470
A Meningococcal Factor H Binding Protein Mutant That Eliminates Factor H Binding Enhances Protective Antibody Responses to Vaccination

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Certain pathogens recruit host complement inhibitors such as factor H (Fh) to evade the immune system. Microbial complement inhibitor-binding molecules can be promising vaccine targets by eliciting Abs that neutralize this microbial defense mechanism. One such Ag, meningococcal factor H-binding protein (FHp), was used in clinical trials before the protein was discovered to bind Fh. The potential effect of Fh binding on vaccine immunogenicity had not been assessed in experimental animals because FHp binds human Fh specifically. In this study, we developed a human Fh transgenic mouse model. Transgenic mice immunized with FHbp vaccine had 4- to 8-fold lower serum bactericidal Ab responses than those of control mice whose native Fh did not bind the vaccine. In contrast, Ab responses were unimpaired in transgenic mice immunized with a control meningococcal group C polysaccharide–protein conjugate vaccine. In transgenic mice, immunization with an Fh nonbinding mutant of FHbp elicited Abs with higher bactericidal activity than that of FHbp vaccination itself. Abs elicited by the mutant FHbp more effectively blocked Fh binding to wild-type FHbp than Abs elicited by FHbp that bound Fh. Thus, a mutant FHbp vaccine that does not bind Fh but that retains immunogenicity is predicted to be superior in humans to an FHbp vaccine that binds human Fh. In the case of mutant FHbp vaccination, the resultant Ab responses may be directed more at epitopes in or near the Fh binding site, which result in greater complement-mediated serum bactericidal activity; these epitopes may be obscured when human Fh is bound to the wild-type FHbp vaccine. The Journal of Immunology, 2011, 186: 000–000.

Surface-exposed proteins from bacterial pathogens are potential vaccine candidates when they are targets of complement-dependent bacterial or opsonophagocytic Abs. Bacterial surface proteins that also bind host complement inhibitors (or complement downregulators) are particularly attractive as vaccine candidates (1, 2) because Abs directed against them may also block binding of the complement inhibitors. Binding of these inhibitors allows certain bacterial species to evade a host innate immune defense that would otherwise result in death of the organism. Downregulation of complement activation occurs when the complement inhibitors are in close proximity to active complement components that are located nearby on the bacterial surface, thereby permitting the organism to disarm a key component(s) of innate host defense and cause disease. Factor H (Fh) is one such important complement inhibitor. A soluble-phase inhibitor of the alternative pathway of complement, Fh inhibits the assembly of an active C3 convertase by competing with factor B for C3b binding, accelerating the decay of the alternative pathway C3 convertase (C3b,Bb), while also acting as a cofactor in factor I-mediated cleavage of C3b to iC3b (3–7). Recently, genetic variation in the human Fh gene cluster was found to affect susceptibility to developing meningococcal disease (8).

The vaccine potential of a number of microbial proteins that bind inhibitors of complement is an active area of investigation. These include, for example, M-protein (Streptococcus pyogenes) (9, 10), OspE (Borrelia burgdorferi) (11), PspC (Streptococcus pneumoniae) (12, 13), and GNA1870 (Neisseria meningitidis); the latter is also known as factor H-binding protein (FHp) (14). A vaccine that elicits Abs directed at bacterial ligands for complement inhibitors, such as Fh, could confer protection by dual mechanisms: 1) by binding of the Abs to the surface of the pathogen, which results in direct activation of complement, and 2) by blocking binding of the complement inhibitor (e.g., Fh), thereby enhancing susceptibility of the pathogen to complement-mediated bactericidal or opsonic activity.

N. meningitidis is a major cause of bacterial meningitis and sepsis worldwide. The organism binds both Fh and C4BP to its surface (14–16), although maximum C4BP binding is observed only under conditions of low stringency, which may limit its physiological role (15). When Fh is bound to the meningococcal cell surface, the ability of Fh to downregulate complement activation enables the organism to survive in human serum or blood (16–18). Ligands for Fh binding to meningococci include a surface-exposed lipoprotein referred to as FHp and a second recently described receptor protein, Neisserial surface protein A (NspA) (19). NspA may be important for evasion of complement-mediated killing by strains with low FHp expression.
Molecular mass determination in comparison with dilutions of a human reference serum that contained 471 2/membrane-bound human fH. Recombinant fHbp (2 µg/ml) was adsorbed with 600 µg of human IgG-depleted fraction exhibited a 30% decrease in hemolytic complement activity measured using diphtheria CRM197 (List Biological Laboratories, Campbell, CA) and group C meningococcal polysaccharide conjugated to adipic dihydrazide (33) as the Ag on the plate, respectively. Serum bactericidal Ab responses were measured using log-phase bacteria and human serum depleted of IgG as a complement source as previously described (34). Briefly, a 1:1 aliquot of human serum was passed over a protein G column (Pierce, Hilden, Germany) and was used to test whether the type of fHbp vaccine and human fH interacted in a binding assay. Recombinant fHbp Ags are part of two promising group B meningococcal vaccines that are in late-stage clinical development (20). These vaccines elicited serum bactericidal Ab responses in mice (21–23) and humans (24–26). It was only after fHbp vaccines had been developed and tested in clinical trials that the Ag was discovered to bind to fH (14). Furthermore, binding of fH to fHbp was found to be specific for human fH (27). A potential undesirable consequence of targeting an Ag that binds a human complement inhibitory protein is formation of a complex between the vaccine Ag and complement protein, which might interfere with Ag presentation and protective Ab responses.

The purpose of the current study was to investigate fHbp immunogenicity under conditions where human fH was present; to accomplish this, we developed a human fH transgenic mouse model. We also investigated the immunogenicity of a newly identified mutant fHbp vaccine containing a single amino acid substitution that eliminated fH binding to mutant fHbp but that retained immunogenicity in wild-type mice. By eliminating fH binding, we hypothesized that in human fH transgenic mice, the mutant fHbp vaccine would elicit Abs with greater potential for protection (e.g., greater bactericidal activity) than that of Abs elicited by wild-type fHbp.

Materials and Methods

Generation of human fH transgenic mice
Full-length CDNA encoding human fH (3.9 kbp) was subcloned into the EcoRI site of the expression vector pCAGGS (28). A CMV enhancer and chicken β-actin promoter sequences are located upstream of the EcoRI site in pCAGGS-human fH (37). A rabbit β-globin polyA sequence is located downstream of the EcoRI site. The resulting plasmid, pCAGGS-human fH, was digested with SalI and PstI to isolate the transgenic cassette fragment consisting of the CMV enhancer, the chicken β-actin promoter, the human fH cDNA, and the rabbit β-globin polyA (Sequence located). The isolated ~9 kb SalI and PstI fragment was purified for microinjection into mouse embryos from BALB/c mice. Mouse embryos were implanted into pseudo-pregnant female BALB/c mice (Charles River Breeding Laboratories) at the Transgenic Facility of the University of Massachusetts Medical School. Human fH transgenic mice initially were identified by PCR analysis using genomic DNA prepared from mouse tails. A region inside human fH was amplified by PCR using primers 5′-CATCTGCTGACTGCTCTTCCAAAA-3′ and 5′-ATCTAATTGATCCTGATGTTTCACC-3′ to yield a 232-bp fragment. Amplified products were electrophoretically resolved in 2% TAE agarose gels and were visualized by ethidium bromide staining under UV light. Expression of human fH in sera of pups was detected by Western blotting using affinity purified goat anti-human fH (Complement Technology, Tyler, TX).

Serum human fH concentrations
To distinguish human from mouse fH, we used an fHbp capture ELISA that specifically binds human fH. Recombinant fHbp (2 µg/ml) in sterile PBS was adsorbed to wells of microtiter plates. After blocking with 1% BSA, dilutions of preimmune mouse (wild-type or transgenic) or human sera were added. Bound human fH was detected using sheep anti-human fH (Lifespan Biosciences, Seattle, WA). Human fH concentrations were determined in comparison with dilutions of a human reference serum that contained 471 µg/ml. Wild-type mice had no detectable serum human fH (<12 µg/ml). For comparison with humans, fH was measured in stored sera from 25 adult subjects in the San Francisco Bay Area who had participated in a protocol approved by the Children’s Hospital Oakland Institutional Review Board to screen normal sera as complement sources for serum bactericidal assays. Recombinant fHbp

The gene encoding fHbp ID 1 (http://pubmlst.org/Neisseria/fHbp) was subcloned into expression plasmid pET21b as described previously (21–23). A R41S substitution was introduced by changing the codon AGG to AGC with the wild-type fHbp vaccine was not lower than those of immunized with wild-type fHbp, which was justified based on the lower responses in transgenic mice observed in Study 1. General linear regression models were used to test whether the type of fHbp vaccine and human fH
concentrations affected serum bactericidal Ab responses. In addition, we calculated the Pearson correlation coefficients between log_{10} serum bactericidal titers and log_{10} human fH concentrations of transgenic mice immunized with the fHbp that bound human fH and the mutant vaccine that did not bind human fH. The test of equality of two Pearson correlation coefficients drawn from the two different samples was performed as described (42). To meet normality assumption, both serum bactericidal Ab measurements and fH concentrations were log_{10} transformed in regression and correlation analyses. A two-tailed p value ≤ 0.05 was considered statistically significant.

Results

A mutant fHbp with a single amino acid substitution, R41S, does not bind human fH

Based on a published crystal structure of fHbp bound to a fragment of fH (43), the arginine residue at position 41 forms a charged hydrogen-bond with fH (Fig. 1A, R41). We predicted that fH binding might be eliminated if the arginine were replaced by serine (Fig. 1A, S41). Because most of the important fHbp epitopes that elicit bactericidal Ab have been reported to be in the region encompassing residues 100 to 255 (44), we speculated that opes that elicit bactericidal Ab have been reported to be in the region encompassing residues 100 to 255 (44), we speculated that fHbp responses to the mutant fHbp. The purified wild-type and R41S mutant proteins showed similar purity and molecular mass by SDS-PAGE (Fig. 1B).

By ELISA, the R41S mutant did not bind human fH (Fig. 2A), but a control anti-fHbp mAb, JAR 4 (31, 32), bound almost identically to both the mutant fHbp and wild-type fHbp (Fig. 2B). This control indicated that comparable amounts of the respective proteins were adsorbed to the wells of the microtiter plate. Further, the R41S substitution did not affect expression of the respective fHbp epitopes involving amino acid residues 121 and 122 (32, 41) (data not shown).

In surface plasmon resonance experiments, we tested binding of soluble fHbp to immobilized human fH, which was performed as described in Materials and Methods. In the first experiment, 2400 response units (RU) of purified human fH were coupled to the biosensor chip; the R41S mutant protein (0.5 μM) showed no binding with fH (−0.6 RU) compared with +22.5 RU with 0.5 μM of the respective native fHbp Ag. As previously reported, the equilibrium dissociation constant for binding of the native fHbp was 45 ± 9 nM (18). In the second experiment, 4000 RU purified human fH was coupled to the biosensor chip; binding of soluble wild-type fHbp (0.5 μM) showed +77.4 RU, whereas 0.5 or 1.0 μM of the mutant R41S fHbp showed no binding (−1.0 or −0.8 RU, respectively). When the concentration of R41S was increased to 2.5 or 5.0 μM, some binding with the mutant was evident (Fig. 2C), which appeared to be nonspecific based on the shape of the association signal and rapid dissociation.

To test the structural integrity of the mutant protein, we determined its thermal stability by differential scanning calorimetry (Fig. 2D). As previously described (30, 45), the wild-type fHbp unfolded with two transitions, with midpoints (T_m) at 69.0 and 84.1˚C (Fig. 2D, black solid line), which corresponded with the respective N- and C-terminal domains. The R41S mutant unfolded with two transitions, centered at 67.9 and 84.7˚C (Fig. 2D, red dashed line). The first transition with the mutant protein was lower than that with the wild-type protein (ΔT_m = −1.1˚C), which was consistent with the location of the amino acid substitution in the N-terminal domain.

The R41S mutant fHbp has preserved immunogenicity in wild-type mice

In wild-type mice whose serum fH did not bind to either vaccine Ag, the wild-type and R41S mutant fHbp vaccines elicited similar serum bactericidal Ab responses (reciprocal GMTs of 77 and 71, respectively; p = 0.32 by t test) (Fig. 3, Initial Study). In contrast, a separate mutant fHbp vaccine, E218A/E239A, which previously

![FIGURE 1](http://www.jimmunol.org/)

A. Structural model of fHbp bound to a fragment of fH based on published atomic coordinates (43). The blue and green ribbons represent the respective N- and C-terminal domains of the fHbp molecule. The gray ribbon represents the sixth and seventh short consensus repeat domains of human fH previously shown to mediate the interaction of human fH and fHbp (43). The arginine residue at position 41 formed a charged H-bond with fH (left lower inset), which was predicted to be eliminated when arginine was replaced by serine (right lower inset). B. SDS-PAGE of fHbp. Wild-type (WT) fHbp and R41S mutant fHbp (5 μg/lane) were separated on 4–12% polyacrylamide gradient gel (NuPAGE; Invitrogen) and stained with Coomassie blue. The molecular masses (in kDa) of the protein standards (Std) are labeled on the left.
A conformational epitope in the N-terminal domain was retained. Symbols two proteins were adsorbed to the wells of the microtiter plate and that solid-phase WT or R41S mutant fHbp indicated that similar amounts of the fH to solid-phase mutant fHbp.

Substitution (red triangles, dashed line) eliminated binding of soluble human fHbp wild type (WT) (blue circles) bound fH, whereas the R41S substitution fHbp to immobilized human fH as measured by surface plasmon resonance buffer data were subtracted as a baseline, and the data were normalized based on the calculated molecular mass of the recombinant fHbp solutions (0.5 mg/ml) were in PBS, and the scan rate was 60˚C/h. Refer-ence buffer data were subtracted as a baseline, and the data were normalized based on the calculated molecular mass of the recombinant fHbp (27.7 kDa). The lower and higher temperature transitions correspond with the unfolding of the N- and C-terminal domains, respectively (45).

had been reported not to bind to fH (43) and to have impaired immunogenicity in wild-type mice (30), elicited significantly lower bactericidal titers (reciprocal GMT of 9 versus 77 for the wild-type fHbp, p = 0.01 by t test) (Fig. 3, Initial Study). These results confirmed diminished Ab responses from possible loss of epitopes or minor destabilization of the C-terminal domain of the molecule that contained the E218A and E239A substitutions, which is in the region of the molecule reported to contain the majority of the epitopes responsible for generating bactericidal Abs (44). In contrast, the Ab responses of the wild-type mice to the R41S mutant fHbp vaccine indicated that substitution of serine for arginine in the N-terminal domain did not decrease immunogenicity. The similar bactericidal Ab responses to the wild-type and R41S mutant fHbp vaccines were replicated in an independent study (reciprocal GMT of 115 versus 83, p = 0.90) (Fig. 3, Rep-licate Study).

**Generation of human fH transgenic mice**

To test the effect of human fH on the immunogenicity of fHbp vaccines, we generated human fH transgenic mice by microinjection of embryos with a PCR product containing the human fH cDNA (Fig. 4A), which was performed as described in Materials and Methods. Transgenic mice containing human fH in their sera were identified by Western blotting (Fig. 4B). We quantified human fH in transgenic mice using an fHbp capture ELISA (Fig. 4C). Wild-type mice had no detectable serum human fH (<12 µg/ml). The mean ± SD concentration of human fH in the sera of the transgenic mice was 268 ± 128 µg/ml. The mean fH concentration ± SD in the human sera was 284 ± 442 µg/ml. The respective means were not significantly different (p = 0.57), but the variance of the fH concentrations of the human sera was lower than in transgenic mice (F = 2.89; p = 0.008).

**Binding of human fH decreases the immunogenicity of fHbp vaccine**

In two studies, human fH transgenic or wild-type mice were immunized with a recombinant fHbp vaccine that bound human fH. Three weeks after the third injection of vaccine, the serum IgG anti-fHbp Ab responses of the transgenic mice were lower than
fHbp vaccine impaired protective Ab responses. Collectively, the data indicated that binding of human fH to the serum human fH concentration, the lower the bactericidal titer. 

In Study 1, serum human fH concentrations of the transgenic mice were 8-fold lower than those of the wild-type mice, whose fH did not bind the vaccine. In Study 2, transgenic mice immunized with the fHbp vaccine that bound human fH again had lower serum bactericidal Ab responses (reciprocal GMT of 31 versus 115 in wild-type mice, \( p = 0.03 \); Study 2, \( p = 0.025 \)). Comparing respective SBA GMTs of fHbp immunized WT and Tg mice: Study 1, \( p = 0.03 \); Study 2, \( p = 0.05 \). Comparing respective SBA GMTs of fHbp immunized WT and Tg mice: Study 1, \( p = 0.03 \); Study 2, \( p = 0.05 \).

Study 1 did not include a non-fHbp control vaccine. Therefore, we were uncertain whether the lower immunogenicity of the fHbp vaccine in the transgenic mice resulted from binding of the vaccine Ag with human fH or whether there might have been lower serum Ab responses of the transgenic animals to vaccine Ags in general. In Study 2, we included groups of transgenic and wild-type mice immunized with a control meningococcal group C polysaccharide–CRM\(_{197}\) conjugate vaccine. The respective serum IgG anti-CRM and anticapsular Ab titers and serum bactericidal Ab responses of the transgenic mice immunized with the meningococcal conjugate vaccine were not significantly different from those of the wild-type mice (Fig. 7). These results indicated that the transgenic mice responded normally to the control vaccine and that the lower responses of transgenic mice to the fHbp vaccine resulted from human fH binding to the vaccine.

FIGURE 5. SBA responses of wild-type (WT; \( X \) symbols) or human fH transgenic (Tg; square symbols) mice immunized with an fHbp vaccine that bound human fH. A, IgG anti-fHbp responses. Titers of individual mice are represented by the respective symbols, and the GMTs are indicated by horizontal lines. The symbols for mice immunized with negative control vaccines are titers of serum pools, each from three to four mice. B, SBA responses measured with human complement against group B strain H44/76. Symbols represent titers of individual mice as in A. Comparing respective IgG GMTs of fHbp immunized WT and Tg mice: Study 1, \( p = 0.03 \); Study 2, \( p = 0.025 \). Comparing respective SBA GMTs of fHbp immunized WT and Tg mice: Study 1, \( p = 0.03 \); Study 2, \( p = 0.05 \).

A mutant fHbp that does not bind human fH has increased immunogenicity in human fH transgenic mice

In Study 2, human fH transgenic mice were immunized with the R41S mutant fHbp that did not bind fH. The IgG anti-fHbp titers were not significantly different from those of control transgenic mice immunized with the wild-type fHbp that bound fH (GMT of 1:106,000 versus 1:105,000; Fig. 8A). The serum bactericidal responses to the mutant R41S fHbp vaccine were ~3-fold higher than those to the fHbp vaccine that bound fH (Fig. 8B), but the difference was not statistically significant (GMT of 96 versus 31, \( p = 0.11 \) by \( t \) test). This analysis, however, did not take into consideration a possible confounding effect of the human fH serum concentrations of the transgenic mice on serum anti-fHbp bactericidal responses. In transgenic mice immunized with the mutant fHbp that did not bind fH, there was no significant cor-
relation between the serum bactericidal Ab responses and serum human fH concentrations ($r = +0.17; p = 0.58; \text{Fig. 6B}$), whereas as described above, there was an inverse correlation with the bactericidal titers elicited by the wild-type vaccine that bound fH ($r = -0.65; p = 0.02; \text{Fig. 6A}$). The respective correlation coefficients for the two vaccines were significantly different ($p = 0.03$).

By general linear regression, there was a significant interaction between the type of fHbp vaccine and the human fH concentration on the bactericidal response (likelihood ratio test, $p = 0.018$). To determine if the type of fHbp vaccine (fHbp wild-type or R41S mutant) affected the serum bactericidal Ab responses of the transgenic mice, we used the regression model to estimate ratios of the reciprocal serum bactericidal GMT of transgenic mice immunized with the R41S mutant vaccine over those of transgenic mice immunized with the fHbp vaccine that bound human fH at various serum human fH concentrations (Fig. 9). Whereas there were no significant differences in bactericidal responses when serum human fH concentrations were low ($<250 \mu g/ml$), the bactericidal responses to the R41S mutant vaccine were significantly higher when the serum fH concentrations were higher than $250 \mu g/ml$ ($p < 0.05$), or higher than $316 \mu g/ml$ ($p < 0.01$). Because many humans have fH concentrations in this range (Fig. 4C), the results suggested that mutant fHbp molecules that do not bind fH might be superior vaccines in humans.

**Abs elicited by the R41S mutant fHbp vaccine have superior inhibition of binding of fH to the nominal vaccine, fHbp**

Binding of human fH to a fHbp vaccine may occlude epitopes on the vaccine Ag that may be necessary to interact with immune cells that influence production of anti-fHbp Abs with functional activity.

To test this hypothesis, we measured inhibition of binding of fH to fHbp by different dilutions of postimmune sera from transgenic mice (Fig. 10A). At 1:100 and 1:400 dilutions, the sera from mice immunized with the fHbp R41S mutant showed greater inhibitory activity than sera from mice immunized with the fHbp that directly bound fH ($p = 0.03$). This greater inhibition in the R41S mutant vaccine group was observed despite the fact that the IgG anti-fHbp Ab responses of the transgenic mice immunized with the two vaccines were similar (Fig. 8A). Collectively, these results indicated qualitative differences in the Ab repertoire elicited by each of the two vaccines. Further, the Abs capable of inhibiting binding of fH appeared to be more protective based on a significant correlation between percentage inhibition of fH binding to fHbp and serum bactericidal titer ($r = 0.69; p = 0.004; \text{Fig. 10B}$).

**Discussion**

In this study, we developed a human fH transgenic mouse model with the intent to investigate the role of this complement downregulator on infections unique to humans where fH has been proposed to play a role ($27, 47, 48$). Human fH, whose amino acid sequence and structure varies from mouse fH, binds uniquely to *Neisseria* spp.; mouse fH does not. We carried this principle...
further and investigated the effect of immunization with the \textit{N. meningitidis} outer membrane protein vaccine candidate, fHbp. To our knowledge, this study represents the first report of expression in a mouse of full-length human fH to permit fH direct binding, via short consensus repeat sequences 6 and 7 (49), to a vaccine component made from \textit{N. meningitidis}. Although the alternative pathway inhibitory action of human fH was not directly investigated in these studies, human fH is known to regulate the non-human alternative pathway of complement similar to its action on the respective human pathway (27, 47).

The respective serum Ab responses of the fH transgenic and wild-type mice to the control group C meningococcal conjugate vaccine were similar. These data indicated that the presence of the transgene and/or human fH in the transgenic mice did not impair serum IgG Ab responses to a control capsular polysaccharide or carrier protein Ag or complement-mediated bactericidal activity of the elicited anticapsular Abs. The effect of human fH on decreasing immunogenicity of the fHbp vaccine in the human fH transgenic mice, therefore, was specific for the fHbp vaccine Ag and was observed in two independent studies. Moreover, there was an inverse correlation between the serum bactericidal Ab responses in the transgenic mice immunized with the vaccine that bound human fH and the serum human fH concentrations of individual mice (Fig. 6A), which strengthened the case for a causal relationship between human fH and decreased fHbp vaccine immunogenicity. Finally, a mutant fHbp vaccine that did not bind fH but that, importantly, retained immunogenicity in wild-type mice elicited higher serum IgG Ab responses to the wild-type fHbp vaccine than to the mutant fHbp vaccine (Fig. 8A).

**FIGURE 9.** Effect of serum human fH concentrations on ratio of bactericidal Ab responses elicited by R41S mutant fHbp vaccine versus wild-type (WT) fHbp vaccine. The respective GMT ratios (R41S mutant to WT) were estimated from the general linear regression model (see text), which showed that the effect of fHbp vaccine type differed by serum fH concentration on bactericidal titer (p = 0.018). Based on the regression model, the ratios of the geometric mean bactericidal responses of the group immunized with R41S fHbp vaccine over that of the group immunized with WT fHbp vaccine were significantly greater than 1 (in favor of the mutant fHbp vaccine) for all human fH concentrations >250 \( \mu \)g/ml (p < 0.05) and for >316 \( \mu \)g/ml (p < 0.01).
bactericidal Ab titers in transgenic mice than those of the fHbp vaccine that bound fH. Collectively, these data indicated that binding of the vaccine Ag to this complement protein, fH, impaired the development of protective Ab responses.

The possibility that a mutant fHbp molecule may be a superior vaccine candidate if it did not bind human fH was suggested by Meri et al. (2) and Schneider et al. (43). Based on the crystal structure of fHbp binding to a fragment of fH, Schneider et al. identified two fHbp glutamate residues that were important for fH binding at positions E218 and E239 based on the numbering of the mature fHbp beginning with the lipidated cysteine residue (22, 50). Factor H binding was eliminated when alanine was substituted at these two positions (43). In a subsequent study, an E218A/E239A mutant fHbp vaccine elicited lower serum bactericidal Ab responses in wild-type BALB/c and CD-1 mice compared with those of a native fHbp vaccine (30), a finding that we confirmed in this report. As noted above, residues 218 and 239 are in the region of the fHbp molecule that is also important for eliciting bactericidal Abs (44). Thus, some mutations that eliminate fH binding may occur as a result of structural changes and/or alterations of electrostatic charges of the molecule that decrease protective Ab responses.

Several lines of evidence indicate that the fHbp R41S mutant vaccine described in the current study, which does not bind human fH, may be a superior fHbp vaccine for humans. In two studies in wild-type mice, the respective serum bactericidal Ab responses elicited by the R41S mutant or the wild-type fHbp vaccines were not significantly different. These data indicated that the epitopes required for eliciting bactericidal Abs were preserved in the R41S mutant when tested in a mouse model in the absence of human fH binding to either vaccine. Second, there were no significant differences in the respective serum bactericidal Ab responses to the two fHbp vaccines in human fH transgenic mice with low human fH concentrations (Fig. 9), compared with elicitation of significantly higher serum bactericidal responses only by the mutant fHbp vaccine when serum human fH concentrations were high, but in a range present in many humans (Fig. 4C). Third, despite having elicited similar serum IgG anti-fHbp titers in transgenic mice (Fig. 8A), the R41S mutant fHbp vaccine elicited Abs with greater fH blocking activity than that of Abs elicited by the fHbp vaccine that bound fH (Fig. 10A). We propose that Abs elicited by the mutant “fHbp” vaccine that was no longer able to bind fH were directed at surface-exposed epitopes near the fH binding site, thereby inhibiting fH binding and contributing to higher serum bactericidal titers. In support of this hypothesis was a significant correlation between percentage inhibition of fH binding to fHbp and serum bactericidal titer (r = 0.69; p = 0.004; Fig. 10B).

Collectively, our data indicate that binding of the human complement regulatory protein, fH, to vaccine Ags can decrease protective Ab responses and that a mutant Ag freed of fH binding, in this case by replacing a large, positively charged amino acid with a smaller, more neutral one, may potentially be a superior immunogen in humans. Mutant fHbp vaccines that do not bind fH may also avoid the theoretical safety risk of exposure of the host to neoantigens, in this case in the form of an fH-fHbp immune complex, which may elicit autoreactive Abs to fH or fH bound to cell surfaces. Our findings, therefore, may have broad implications in the development of vaccines against microbes that bind other host molecules as well.

Acknowledgments

We thank Dr. Barbara Brogioni (Novartis Vaccines, Siena, Italy) for conducting the surface plasmon resonance binding experiments and Dr. Anthony Schyvers (University of Calgary) for providing the group C meningococcal conjugate vaccine.

Disclosures

D.M.G. is a principal investigator of laboratory research conducted on behalf of Children’s Hospital Oakland Research Institute, which is funded by grants from Novartis Vaccines and Diagnostics and from Sanofi Pasteur. D.M.G. also holds a paid consultancy from Novartis. P.A.R., S.R., J.S., P.T.B., and D.M.G. are inventors on patents or patent applications in the area of meningococcal B vaccines.

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