A Broadly Cross-Reactive Monoclonal Antibody Against an Epitope on the N-terminus of Meningococcal fHbp

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Meningococcal factor H binding protein (fHbp) is an important vaccine antigen for prevention of disease caused by capsular group B strains. The protein has been sub-classified into three variant groups. Most anti-fHbp antibodies are variant group-specific and recognize epitopes on the C-terminal domain. We report a murine IgG1 mAb, JAR 41, which broadly cross-reacted with fHbp sequence variants from all variant groups. The mAb bound to the surface of live meningococci with fHbp from each of the three variant groups. In combination with second non-bactericidal anti-fHbp mAbs, JAR 41 elicited complement-mediated bactericidal activity in vitro, and augmented passive protection against meningococcal bacteremia in human fH transgenic rats. The epitope was located on a conserved region of the N-terminal portion of the fHbp molecule opposite that of fH contact residues. The data underscore the importance of broadly cross-reactive, surface-exposed epitopes on the N-terminal domain in the design of protective fHbp vaccines.

Results

Anti-fHbp mAb JAR 41 is broadly cross-reactive. By ELISA, JAR 41 had similar concentration-dependent binding to fHbp ID 1 (variant group 1) as that of a control anti-fHbp, JAR 5, which is specific for variant group 1 (Figure 1a). Two other anti-fHbp mAbs, JAR 11 and JAR 35, which bind fHbp from variant groups 2 and 3, but not from group 1, served as negative IgG controls. JAR 41 also showed similar concentration-dependent binding to fHbp ID 77 (variant group 2) and fHbp ID 28 (variant group 3) as that of the respective positive controls, variant group-specific mAbs (Figures 1b and 1c, respectively). There was no reactivity of these two proteins with JAR 5, which was specific for fHbp variant group 1.

As of December 2011, more than 500 unique fHbp amino acid sequence variants had been deposited in the public database, http://pubmlst.org/neisseria/fHbp/. To select sequence variants representative of fHbp diversity
for measuring the breadth of JAR 41 reactivity, we performed a phylogenetic analysis of 531 sequences in the public database in the form of a network diagram produced using the program SplitsTree 4.2 (Figure 2a). Each unique sequence is represented by a “twig” (node), which are distributed on the network into two sub-families, A and B, as described by Fletcher et al.7, or three variant groups, 1, 2 or 3, as described by Masignani et al.12. Sub-family A contains the sequences in variant groups 2 and 3, and sub-family B contains the sequences in variant group 1. The proteins also can be classified into ten modular groups based on the respective lineages of five variable segments13,18.

We selected 21 amino acid sequence variants representative of the known fHbp amino acid sequence diversity for expression of recombinant proteins for testing JAR 41 reactivity (Table 1). This panel included variants from both sub-families A and B, all three variant groups12, and all ten modular groups13,18 (Figure 2b). Note that fHbp ID 283, which is a natural hybrid of variant groups 1 and 3, has A, B, and C segments from lineage 2, and D and E segments from lineage 1. This variant was assigned to a new modular group (X).

JAR 41 showed similar respective concentration-dependent binding with all of the amino acid sequence variants in variant groups 1, 2 and 3 (Figure 3a–c), and with two natural hybrids, ID 207 and ID 283 (Figure 3d). Binding was specific for fHbp in that JAR 41 did not bind to two unrelated recombinant meningococcal surface proteins, NadA and GNA1030 (Figure 3e). Also, with each of the sequence variants tested by ELISA, we included negative control IgG anti-fHbp mAbs that were specific for fHbps from heterologous variant groups, which showed negligible binding to the sequence variant tested for binding with JAR 41 (See for example, Figure 1).

The JAR 41 epitope is exposed on the surface of live meningococci. By flow cytometry, JAR 41 bound to the surface of live bacteria from N. meningitidis strains. Figure 4a compares binding of JAR 41 (25 μg/ml) to wild-type group B strain H44/76 (fHbp ID 1, variant group 1) and an isogenic mutant of strain H44/76 with lower fHbp expression (H44/76-LE). There is ~4-fold lower binding with the mutant strain. There was no detectable binding to either strain with a control IgG mAb, JAR 11 (specific for fHbps in variant groups 2 or 3). Binding of an anti-PorA mAb was similar with both the wild-type and mutant (Figure 4b), which is evidence that similar numbers of wild-type and mutant cells were tested for JAR 41 binding.

We next tested binding of JAR 41 to strains H44/76 and its isogenic mutant using a range of mAb concentrations (Figures 4c and 4d, respectively). As little as 0.2 μg/ml of JAR 41 showed significant binding to the H44/76-WT or -LE mutants over that of background binding, which was determined by testing 25 μg/ml of JAR 41 with a H44/76 fHbp knockout mutant (light gray shaded area). JAR 41 also bound to the surface of group B strains 8047 (fHbp ID 77, variant group 2) and M1239 (fHbp ID 28, variant group 3) (Figures 4e and 4f, respectively). For each of these strains the respective JAR 41 binding was similar when tested at the two highest concentrations (5 μg/ml or 25 μg/ml for 8047, or 5 and 50 μg/ml for M1239 [grey-shaded area and solid lines, respectively]). Strain 8047 also was tested with lower concentrations of the mAb. Binding above that of the IgG negative control (an IgG anti-fHbp mAb specific for variant group 1) was detected with as little as 0.2 μg/ml of JAR 41.
that the respective IgG Fc regions can engage C1q24. When an binding of two IgG mAbs to appropriately spaced epitopes such mutant [Figure 5b], 8047 [Figure 5c] or M1239 [data not shown]. of the four test strains (H44/76-WT [Figure 5a], the H44/76 LE tested individually at concentrations of up to 100 \( \mu \text{g/ml} \) with second anti-fHbp mAbs JAR 41 elicits human complement-mediated bactericidal activity of human fH to the surface of meningococci down-regulates JAR 41 augments passive protective activity of anti-fHbp mAb JAR 41 against experimental meningococcal bacteremia. JAR 41 elicits human complement-mediated bactericidal activity with second anti-fHbp mAbs. JAR 41 was not bactericidal when tested individually at concentrations of up to 100 \( \mu \text{g/ml} \) against any of the four test strains (H44/76-WT [Figure 5a], the H44/76 LE mutant [Figure 5b], 8047 [Figure 5c] or M1239 [data not shown]). Activation of the classical complement pathway by IgG requires binding of two IgG mAbs to appropriately spaced epitopes such that the respective IgG Fc regions can engage C1q2. When an individual mAb binds to a sparse antigen, there may be insufficient immune complex formed to activate bacteriolysis, whereas combinations of more than one mAb may be sufficient3. When tested in combination with other non-bactericidal anti-fHbp mAbs specific for fHbp in variant group 1, JAR 41 elicited cooperative bactericidal activity against strains H44/76 (Figure 5a) or the H44/76 LE mutant with lower fHbp expression (Figure 5b). In contrast, JAR 41 was not bactericidal in combination with anti-fHbp mAbs specific for variant groups 2 or 3 when tested against strains 8047 (fHbp variant group 2, Figure 5c) or M1239 (fHbp variant group 3, data not shown). Both of these strains were susceptible to bactericidal activity elicited by anti-fHbp mAb JAR 4 when tested with the anti-fHbp mAbs specific for variant groups 2 or 3 (for example, JAR 4 + JAR 11 or JAR 13 against strain 8047, Figure 4c).

JAR 41 augments passive protective activity of anti-fHbp mAb JAR 5 against experimental meningococcal bacteremia. Binding of human fH to the surface of meningococci down-regulates complement activation, which enables the organism to evade host defenses29–31. Binding is specific for human fH32. We recently developed a human fH transgenic infant rats with 25 \( \mu \text{g} \) of JAR 5 conferred partial protection against bacteremia, as evidenced by a lower geometric mean CFU/ml in blood obtained 6 hrs after IP challenge, compared to that of control rats that received PBS \((p<0.05, \text{Experiments 1 and 2, Figure 6)}). In contrast, 25 \( \mu \text{g} \)/rat of JAR 41 did not confer significant protection in either experiment \((p>0.05)\). To determine whether JAR 41 could augment JAR 5 protective activity, both antibodies \((12.5 \mu \text{g/rat} \text{of each mAb})\) were given in combination to groups of human fH transgenic infant rats. In both experiments, none of these animals had bacteremia 6 hrs after the IP challenge. In Experiment 2, in which we tested larger numbers of animals, the augmentation of protection by the combination of JAR 41 and JAR 5 was statistically significant \((6 \text{ of } 9 \text{ with bacteremia in the JAR 5 or JAR 41 alone groups, vs. } 0 \text{ of } 8 \text{ in the JAR 5+JAR 41 combination group, } p=0.009)\).

JAR 41 recognizes an epitope that overlaps that of anti-fHbp mAb JAR 4. While the combination of JAR 4 with JAR 5, or JAR 4 with mAb502, was bactericidal against group B strain H44/76, JAR 41 was not bactericidal in combination with JAR 4 \((\text{Figure 5a)}\). One possible explanation was that JAR 4 and JAR 41 recognized overlapping epitopes. To investigate this question, we measured the ability of JAR 4 to inhibit binding of alkaline phosphatase (AP)-conjugated JAR 41 to fHbp (Supplemental Figure S1a). The positive control, unconjugated JAR 41, gave \(>95\%\) inhibition of binding while the negative control, unconjugated JAR 5, gave \(<5\%\) inhibition. At the highest concentration of mAb tested \((100 \mu \text{g/ml})\), JAR 4 gave \(\sim 70\%\) inhibition of binding of AP-JAR 41 to fHbp. We also measured the

| Source Strain (fHbp gene) | fHbp ID1 | Sub-family2 | Variant Group3 | Modular Group4 | Control Anti-fHbp mAb Reactivity5 |
|---------------------------|---------|------------|---------------|---------------|----------------------------------|
| MC58                      | B       | 1          | I             |               | +                                |
| Synthesized7               | 2       | B          | 1             |               | +                                |
| SK068                     | 4       | B          | 1             |               | +                                |
| M6190                     | 6       | B          | 1             |               | +                                |
| Mali 29/07                 | 9       | B          | 1             |               | +                                |
| SK105                     | 13      | B          | 1             |               | +                                |
| NZ98/254                  | 14      | B          | 1             |               | +                                |
| Uganda 6/07                | 74      | B          | 1             |               | +                                |
| NM452                     | 15      | B          | 1             |               | +                                |
| CDC:1573                  | 55      | B          | 1             |               | +                                |
| RM1090                    | 22      | A          | 2             |               | +                                |
| SK139                     | 19      | A          | 2             |               | +                                |
| 8047                      | 77      | A          | 2             |               | +                                |
| M1239                     | 28      | A          | 3             |               | +                                |
| 2040                      | 111     | A          | 3             |               | +                                |
| NZ77/00                   | 45      | A          | 3             |               | +                                |
| S3032                     | 79      | A          | 3             |               | +                                |
| MA:5756                   | 67      | A          | 3             |               | +                                |
| 19498                     | 175     | A          | 3             |               | +                                |
| 0167/03                   | 207     | A/B        | 1/2           |               | +                                |
| Synthesized7               | 283     | A/B        | 1/3           |               | +                                |

1Amino acid sequence variant in the public database, http://pubmlst.org/neisseria/fHbp/.
2Sub-family as described by Murphy et al11.
3Variant group as described by Masignani et al12.
4Modular groups as described by Beemink and Grandal13 and Pagan et al14.
5Positive reactivity, OD = 0.3 when tested at 0.1 \( \mu \text{g/ml} \); negative reactivity, OD = 0.0 when tested at 1 \( \mu \text{g/ml} \).
6JAR 4 to inhibit binding of alkaline phosphatase (AP)-conjugated JAR 41 to fHbp (Supplemental Figure S1a). The positive control, unconjugated JAR 41, gave \(>95\%\) inhibition of binding while the negative control, unconjugated JAR 5, gave \(<5\%\) inhibition. At the highest concentration of mAb tested \((100 \mu \text{g/ml})\), JAR 4 gave \(\sim 70\%\) inhibition of binding of AP-JAR 41 to fHbp. We also measured the

ability of unconjugated JAR 41 (IgG1) to inhibit binding of unconjugated JAR 4 (IgG2a) using an AP-conjugated secondary antibody that was specific for IgG2a (Supplemental Figure S1b). JAR 41 gave >95% inhibition of binding of JAR 4 to fHbp. The ability of JAR 41 and JAR 4 to inhibit binding of each other to fHbp implied that the respective epitopes overlapped.

The fHbp epitope recognized by JAR 41 is eliminated by substitution of Ala for Asp at residue 25 in fHbp. We used a mutant fHbp ID 1 library displayed on the surface of yeast to identify amino acid residues that potentially affect JAR 41 binding. The yeast library was sorted to select yeast clones that had lost their ability to bind JAR 41, but which retained their ability to bind JAR 3 (which verified surface-expression of the construct). Individual JAR 41-negative/JAR 4 positive yeast colonies were expanded and the recombinant mutant proteins were sequenced. After eliminating sequences containing large deletions or multiple amino acid substitutions, we aligned the predicted amino acid sequences of 13 full-length mutant fHbp genes (Supplemental Figure S2). The mutations that abolished JAR 41 binding in the yeast library included three substitutions for Asp at position 25 (D25G, D25H, D25A), one substitution for Lys at position 27 (K27N), and one substitution for His at position 26 (H26N, which was present in combination with D25A). Substitutions at residue K27 and, to a lesser extent, D25A, corresponded to the region of the N-terminal domain also are highly conserved.

The respective locations were consistent with ELISA data showing that neither JAR 41 nor JAR 41 mAb inhibited binding of fH to fHbp (data not shown). JAR 41 individually did not elicit human complement-mediated bactericidal activity, which is typical of most reported murine anti-fHbp mAbs15,16,24,29. fHbp is relatively sparsely distributed15, and it is possible that the distance between most of the fHbp molecules on the bacterial surface exceeds that required for optimal engagement of the N-terminal domain15 of JAR 41.
C1q by binding of individual mAbs. In contrast, binding of two IgG antibodies that recognize appropriately spaced non-overlapping epitopes may permit more efficient engagement of C1q and activation of classical complement pathway bacteriolysis. Since binding of fH to N. meningitidis increases resistance of the organism to complement-mediated killing, the ability of at least one of the anti-fHbp mAbs in the pair to inhibit fH can increase mAb bactericidal activity only against strain H44/76 with fHbp in variant group 1, and an isogenic mutant, H44/76-LE with lower fHbp ID 1 expression (Figure 5). The amount of expression of fHbp variant group 1 by the mutant appeared to be similar to that of the two strains with fHbp in variant groups 2 or 3 that were resistant to anti-fHbp JAR 41 cooperative bactericidal activity. Most likely the resistance of these two test strains reflected testing bactericidal activity with different second anti-fHbp mAbs, which were specific for fHbp in variant groups 2 or 3, than those used to test the strains with fHbp variant group 1. Conceivably, different proximity and/or spatial orientations of JAR 41 bound in combination with JAR 5 or mAb502 in strain H44/76, as
compared with JAR 11 or 13 in strain 8047, may have permitted more efficient engagement of C1q and activation of the classical complement pathway with strain H44/76.

In the present study, we used a recently described human fH transgenic infant rat model to investigate the ability of JAR 41, individually or in combination with JAR 5, to confer passive protection against meningococcal bacteremia caused by group B strain H44/76. We chose to use the transgenic infant rat model because binding of fH to N. meningitidis is specific for human fH. Thus, measuring the ability of anti-fHbp mAbs to confer passive protection in the transgenic model likely provided information relevant to protection in humans where bound human fH can down-regulate complement activation on N. meningitidis.

Passive administration of JAR 5 conferred partial protection against meningococcal bacteremia in the transgenic rats (Figure 6). This protective activity was of interest since JAR 5 individually did not elicit human complement-mediated bactericidal activity but in previous studies was shown to block binding of human fH to fHbp. In contrast, JAR 41, which was not bactericidal and which did not block fH binding, was not protective against bacteremia in the transgenic rat model. JAR 41, however, augmented protective activity of JAR 5 (sterile blood cultures in all the rats given the combination). This result was consistent with the ability of JAR 41 to elicit cooperative complement-mediated bactericidal activity when tested with JAR 5.

Figure 5 | Cooperative bactericidal activity elicited by JAR 41, individually or in combination with second anti-fHbp mAbs. Panel a, strain H44/76 (fHbp ID 1, variant group 1). Panel b, mutant of strain H44/76 engineered to have lower expression of fHbp ID 1 as that of the wild-type strain (See Figure 4a). Panel c, strain 8047 (fHbp ID 77, variant group 2). BC50%, concentration of mAb (µg/ml) resulting in 50% decrease in CFU/ml after 1 hr incubation with human complement, compared to time 0. The combinations contained 1:1 mixtures (i.e., BC50% of 1 µg/ml contained 0.5 µg/ml of each of the mAbs in the combination). The anti-capsular mAb (SEAM 12) or anti-PorA mAbs (P1.7, panels a and b; or P1.2, panel c) served as positive controls. Error bars represent range of results from two to three independent assays.

Figure 6 | JAR 41 augments passive protective activity of anti-fHbp mAb JAR 5 against bacteremia caused by group B strain H44/76 in human fH transgenic infant rats. Panel a, Experiment 1, 8- to 9-day old rats challenged IP with 4900 CFU/rat; Panel b, Experiment 2, 6- to 7-day old rats challenged IP with 760 CFU/rat. In both experiments, rats were given a total dose of 25 µg of mAb or phosphate buffered saline alone (PBS, negative control) 1 hr before the bacterial challenges. The combination contained 12.5 µg of each mAb. Blood cultures were obtained 6 hrs after challenge. Compared to PBS, rats given JAR 5 alone had a lower geometric mean CFU/ml (Experiment 1, p=0.04 and Experiment 2, p<0.03) but there was no protection by JAR 41 alone (p>0.05). In Experiment 2, the combination of JAR 41 and JAR 5 (0/8 with bacteremia) had greater protective activity than JAR 5 alone (6 out of 9 with bacteremia, p=0.009, Fisher’s exact test). ND, not done.

Figure 7 | Effect of amino acid substitutions on binding of mAbs to recombinant fHbp.

Figure 8 | Cooperative bactericidal activity elicited by JAR 41, individually or in combination with second anti-fHbp mAbs. Panel a, strain H44/76 (fHbp ID 1, variant group 1). Panel b, mutant of strain H44/76 engineered to have lower expression of fHbp ID 1 as that of the wild-type strain (See Figure 4a). Panel c, strain 8047 (fHbp ID 77, variant group 2). BC50%, concentration of mAb (µg/ml) resulting in 50% decrease in CFU/ml after 1 hr incubation with human complement, compared to time 0. The combinations contained 1:1 mixtures (i.e., BC50% of 1 µg/ml contained 0.5 µg/ml of each of the mAbs in the combination). The anti-capsular mAb (SEAM 12) or anti-PorA mAbs (P1.7, panels a and b; or P1.2, panel c) served as positive controls. Error bars represent range of results from two to three independent assays.
In summary, JAR 41 is a new murine IgG1 mAb that recognizes a broadly cross-reactive, surface-exposed epitope on the N-terminal domain of fHbp from variant groups 1, 2 and 3. The mAb elicits cooperative human complement-mediated bactericidal activity in combination with other non-bactericidal anti-fHbp mAbs, and augments passive protective activity against meningococcal bacteremia. The JAR 41 epitope overlapped that of JAR 4, which also had cooperative bactericidal activity but was specific for fHbp from variant groups 1 and 2. Collectively, the data demonstrate the importance of including cross-reactive epitopes in N-terminal domain of fHbp in the design of optimally protective fHbp vaccines.

Methods
Production of anti-fHbp mAB JAR 41. A human fH transgenic BALB/c mouse was immunized with three injections of a recombinant fHbp vaccine as part of a previous study37. The fHbp sequence variant was from variant group 1 and assigned ID 1 as designated in the public fHbp peptide database at http://pubmlst.org/neisseria/fHbp/. The mouse received three injections of the vaccine, IP, along with aluminum hydroxide adjuvant followed by a fourth injection without adjuvant. Splenocytes were isolated three days later for fusion to the mouse myeloma cell line P3X63Ag8.653 using established methods14. Cells were plated into 96-well plates. After two weeks of incubation, cell culture supernatants were tested for reactivity to recombinant fHbp ID 1 by ELISA (see below). Cells from positive wells were expanded, cloned by limiting dilution, and the supernatants confirmed for reactivity to fHbp. The cloning was repeated 2 times to ensure monoclonality.

Control anti-fHbp mAbs. The following murine anti-fHbp mAbs were used as controls in different binding and functional assays: JAR 1 (IgG3), JAR 3 (IgG3), JAR 4 (IgG2a), JAR 5 (IgG2b)15, and mAb502 (IgG2a) were from mice immunized with fHbp 16 in variant group 1; JAR 11 (IgG2a) and JAR 13 (IgG2a) were from a mouse immunized with fHbp ID 16 in variant group 22, and JAR 31 (IgG2b) (Unpublished data of DMG) and JAR 35 were from a mouse immunized with fHbp ID 28 in variant group 3.

Purification of recombinant fHbp. We prepared 21 different recombinant fHbp amino acid sequence variants from variant groups 1, 2 or 3 (Table 1) as previously described34. The proteins were expressed from the T7 promoter using the E. coli plasmid pET21b (Novagen, Madison, WI) as described previously15. The recombinant proteins were purified by immobilized metal ion chromatography using Ni-NTA agarose (Qiagen, Valencia, CA) and independent mutant clones were verified by DNA sequencing.

Anti-fHbp ELISA. Binding of anti-fHbp mAbs to recombinant fHbp (rFhb) was measured by ELISA, which was performed as previously described34 with minor modifications. In brief, 2 μg/ml of purified recombinant fHbp diluted in PBS was used to sensitize the microtiter plates. After blocking, serial dilutions of the mAbs were added and incubated at 4°C overnight. The wells were washed and bound antibody was detected with goat anti-mouse IgG conjugated with alkaline phosphatase. The ability of JAR 41 to inhibit binding of fH to fHbp was also measured by ELISA as previously described34.

Epitope mapping using yeast display. Randomly mutated sequences of fHbp allele 1 (http://pubmlst.org/neisseria/fHbp/), which encodes fHbp amino acid sequence variant ID 1, were generated by error-prone PCR. In brief, MnCl2 concentrations were titrated and conditions selected such that the average number of nucleotide substitutions per fHbp gene copy resulted in 1 to 3 amino acid substitutions. The fragments were ligated into pAI1 vector (CIaN, added in E. coli, excised, inserted into the yeast expression vector pYDJ1 (Invitrogen, Carlsbad, CA), and transfected into the yeast EBY100 strain (Invitrogen) to form the mutated fHbp expression library. The mutated fHbp peptides were displayed on the surface of yeast as Aga2 fusion proteins34,35. The library was expanded overnight at 30°C, transferred into galactose-containing YNB medium (Yeast Nitrogen Base w/o amino acids [Difco]) to induce recombinant protein expression, and incubated for 48 hrs at 20°C. Bulk yeast cultures were simultaneously stained with anti-fHbp mAb, JAR 41, which was detected with FITC-conjugated antibody to mouse IgG, and a chimeric human IgG1 mouse anti-fHbp mAb, JAR 33, which was detected with DyLight 649-conjugated antibody to human kappa light chain. JAR 3 and JAR 41 recognized non-overlapping epitopes as evidenced by lack of inhibition of binding by ELISA of either fHbp to ID 1 by the other mAb (data not shown). The yeast cultures were sorted by flow cytometry (FACStar, BD) to select yeast clones that had lost their ability to bind JAR 41, but which retained the ability to bind JAR 3 (which verified surface-expression of the construct). The sorted yeast were plated and incubated at 30°C to isolate single colonies. The individual clones were re-grown, their binding profiles verified by flow cytometry, and the sequence of the respective fHbp insert determined to identify amino acid substitutions that had resulted in the loss of the JAR 41 epitope. Approximately 20 JAR 41-negative/JAR 3-positive yeast clones were analyzed and the sequences of their inserts aligned to identify altered residues that led to loss of JAR 41 binding.

Construction of site-specific mutants. Recombinant fHbp mutants with single amino acid substitutions were constructed using the QuikChange II kit (Stratagene, La Jolla, CA) using the manufacturer's protocols as previously described34. The mutagenesis reactions were transformed into chemically competent E. coli DH5α (Invitrogen, Carlsbad, CA) and independent mutant clones were verified by DNA sequencing (Davis Sequencing, Davis, CA).

Flow cytometry. Binding of the murine mAbs to the surface of live encapsulated bacteria was measured by flow cytometry, which was performed as described previously35. The group B test strains were H44/76 (B:15:P1.7,16; ST-32) with fHbp ID 1, which was previously described35, and JAR 31 (B:14:P1.23,14; ST-41/44), which expressed fHbp ID 28 in variant group 3. The bacterial cells were grown in Mueller Hinton broth culture, harvested by centrifugation, washed, and resuspended in buffer as described elsewhere35.

Serum bactericidal assay. Human complement-mediated bactericidal activity was measured as previously described36 using group B strain H44/76, 8047 and M1239 described above. The complement source was serum from a healthy adult who participated in a protocol that was approved by the Children's Hospital Oakland Institutional Review Board (IRB). Written informed consent was obtained from the subject. The serum had normal total hemolytic complement activity and no detectable serum bactericidal antibodies against the test strains. The serum was depleted of IgG using a protein G column (HiTrap Protein G, GE Life Sciences, Piscataway, NJ) as previously described34 to remove non-bactericidal antibodies that might augment anti-fHbp mAb activity.

The bactericidal activity (BIComp) was defined by the mAb concentration that resulted in a 50% decrease in CFU/ml after 60-min incubation in the reaction mixture compared with CFU/ml in negative control wells at time zero.

Passive protective activity against bacteremia. Details of the human fH transgenic rat meningococcal bacteremia model have been recently described37. For measurement of passive protective activity, at time 0 human fH transgenic rats, ages 8 to 9 days (experiment 1) or 6 to 7 days (experiment 2), were administered 25 μg IP of anti-fHbp mAb JAR 5 or JAR 41, or a combination of JAR 5 + JAR 41 (12.5 μg of each). Control rats received buffer alone (phosphate buffered saline, PBS) or 12.5 μg of an anti-2A mAb (P17,1; exposure 1 only). Total volume of mAb or buffer for each animal was 100 μl. At 1.5 hrs, animals were challenged IP with 4900 CFU (experiment 1) or 760 CFU (experiment 2) of group B strain H44/76. At 7.5 hrs, blood samples were obtained by cardiac puncture into syringes containing 25 U heparin, 1, 10, and 200 μl aliquots of each blood sample were spread onto chocolate agar plates, which were then incubated overnight at 37°C in 5% CO2. Colony counts were obtained the following day to determine CFU/ml. The lower limit of detection was 5 CFU/ml.

Statistical analyses. Statistical analyses were performed using Prism for Mac version 5.0d (GraphPad Software, La Jolla, CA). Comparisons of the proportions of rats that developed bacteremia after pre-treatment with different mAbs or PBS were performed using the Fisher exact test. For the purpose of calculating the geometric mean CFU/ml of blood, sterile blood cultures were assigned a value of half of the lower limit of detection (i.e., <5 CFU/ml was assigned a value of 2.5 CFU/ml). Comparisons of the geometric mean CFU of bacteria per ml of blood between any two groups of rats were performed on log-transformed values using the Student t-test. All probability values reported are two-tailed.

Live vertebrate experiments. All experiments in mice and infant rats were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, U.S.A. The protocols were approved by the Children’s Hospital & Research Center at Oakland Institutional Animal Care and Use Committee. Blood collection was performed under anesthesia, and all efforts were made to minimize suffering.

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Author contributions statement

R.P. performed the phylogenic analyses of fHbp sequence variants and prepared Figure 2 and Supplementary Figure 4. D.C.R. sorted the fHbp ID 1 yeast display mutants and assisted with mapping the JAR 41 epitope. D.M.V. and D.M.G. designed the study, wrote the main manuscript text and prepared the remaining figures and table 1. All authors reviewed and contributed to writing of the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests

D.M.G. holds a paid consultancy with Novartis Vaccines and Diagnostics. D.M.G. R.P. and D.M.V. are inventors on patents or patent applications in the area of meningococcal B vaccines. D.C.R. report no conflicts of interest.

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ERRATUM: A Broadly Cross-Reactive Monoclonal Antibody Against an Epitope on the N-terminus of Meningococcal fHbp

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There are several typographical errors in this Article. In the legend of Figure 7, “(Open triangles with dashed line)” should read, “(Open triangles with solid line)” and, “(Open circles with solid line)” should read, “(Open circles with dashed line)”. In the Results, JAR 41 elicits human complement-mediated bactericidal activity with second anti-fHbp mAbs subsection, “(for example, JAR 4 or JAR 11 or JAR 13 against strain 8047, Figure 4c)” should read, “(for example, JAR 4 or JAR 11 or JAR 13 against strain 8047, Figure 5c)”. In the JAR 41 recognizes an epitope that overlaps that of anti-fHbp mAb JAR 4 subsection, “While the combination of JAR 4 with JAR 5, or JAR 4 with mAb502” should read, “While the combination of JAR 41 with JAR 5, or JAR 41 with mAb502”.
