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Broad vaccine coverage predicted for a bivalent recombinant factor H binding protein based vaccine to prevent serogroup B meningococcal disease

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ABSTRACT

Factor H binding proteins (fHBP), are bacterial surface proteins currently undergoing human clinical trials as candidate serogroup B *Neisseria meningitidis* (MnB) vaccines. fHBP protein sequences segregate into two distinct subfamilies, designated A and B. Here, we report the specificity and vaccine potential of mono- or bivalent fHBP-containing vaccines. A bivalent fHBP vaccine composed of a member of each subfamily elicited substantially broader bactericidal activity against MnB strains expressing heterologous fHBP than did either of the monovalent vaccines. Bivalent rabbit immune sera tested in serum bactericidal antibody assays (SBAs) against a diverse panel of MnB clinical isolates killed 87 of the 100 isolates. Bivalent human immune sera killed 36 of 45 MnB isolates tested in SBAs. Factors such as fHBP protein variant, PorA subtype, or MLST were not predictive of whether the MnB strain could be killed by rabbit or human immune sera. Instead, the best predictor for killing in the SBA was the level of *in vitro* surface expression of fHBP. The bivalent fHBP vaccine candidate induced immune sera that killed MnB isolates representing the major MLST complexes, prevalent PorA subtypes, and fHBP variants that span the breadth of the fHBP phylogenetic tree. Importantly, epidemiologically prevalent fHBP variants from both subfamilies were killed.

1. Introduction

N. meningitidis is a major cause of bacterial sepsis and meningitis which mainly affects two populations: children under 2 years old and adolescents/young adults [1]. In the latter group, entrance into crowded living situations such as college dormitories or military barracks contributes to an increased disease incidence. The insidious onset and rapid progression of the disease result in mortality rates of approximately 10%, despite the availability of effective antibiotics. Protective immunity to meningococcal disease is provided by humoral immunity, mainly through complement-mediated antibody-dependent bactericidal activity in the serum [2–4]. Of the five major disease-causing serogroups A, B, C, Y and W135, capsular polysaccharide has been an effective target against four (A, C, Y and W135), through immunization with polysaccharide-protein conjugate vaccines [5–9]. However, the challenge remains with *N. meningitidis* serogroup B (MnB), which on average accounts for >50% of meningococcal infections in the United States and in Europe [10–13]. A polysaccharide-protein conjugate based vaccine approach for MnB has been unsuccessful, as the MnB capsular polysaccharide is structurally similar to polysialic acid found on human neural antigens [14,15]. This renders it a poor immunogen, and has raised safety concerns.

The need to develop a universal MnB vaccine targeting other surface antigens led to the identification of the lipoprotein, LP2086. LP2086 was identified through biochemical fractionation of MnB outer membrane protein preparations that elicited PorA-independent bactericidal antibodies capable of killing diverse strains [16,17]. LP2086, also known as GNA1870 [18], is a human

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factor H binding protein (fHBP) that interacts specifically with human factor H, leading to the down-regulation of the alternative complement pathway and thereby enhancing serum resistance of the invading organism [19–21]. The fHBP gene is present in all meningococcal strains examined to date [22]. Based on amino acid sequence variation, the fHBP family of proteins can be divided into two subfamilies (A and B), which exhibit only 60–75% amino acid identity between subfamilies. However, amino acid identity within subfamilies is >83%. Other authors [18] have divided the fHBP family into three groups (v.1, v.2, and v.3). Genetic distances (based on pairwise amino acid identities) between v.1 and either v.2 or v.3, however, are substantially greater (2.5–3-fold) than those between v.2 and v.3 [22]. Recent sequence analysis of >1800 MnB isolates (1263 of which were systematically collected) from meningococcal reference centers in the US and Europe confirmed the two subfamily (A/B) phylogeny of Fletcher et al. [16], and showed that 30% of the isolates contained a subfamily A gene while 70% contained a subfamily B gene [22].

Initial immunogenicity studies using lipidated single variants of recombinant fHBP (rfHBP) as vaccines suggested that protective bactericidal antibody responses were largely subfamily-specific [16]. In the present study, we evaluated the specificity and vaccine potential of mono- or bivalent rfHBP-containing vaccines against a diverse panel of disease-causing strains. Rabbit immune sera generated with mono- or bivalent vaccines were tested for breadth of bacterial surface reactivity by flow cytometry, and for bactericidal activity against a panel of diverse MnB clinical isolates. Bivalent rabbit immune sera were tested in SBAs against a panel of 100 invasive MnB isolates. Pooled human sera, from a phase 1 clinical study of a bivalent rfHBP vaccine, were tested in SBAs against a subset of the 100 diverse MnB isolates.

2. Materials and methods

2.1. Serogroup B strains

A set of 100 invasive MnB clinical isolates was selected that represent fHBP, PorA and MLST diversity among invasive MnB isolates. Thirty of the strains were previously characterized with respect to fHBP sequence by Fletcher et al. [16]. The remaining 70 strains were obtained as part of ongoing epidemiological studies to monitor fHBP sequence distribution [22]. Strains were provided by Leonard Mayer, Centers for Disease Control and Prevention, Atlanta, GA (ABCs isolates); Ray Borrow, Health Protection Agency, Manchester, UK; Mohamed-Kheir Taha, Institute Pasteur, Paris, France; Paula Kriz, National Institute of Public Health, Prague, Czech Republic; and Dominique Caugant, Norwegian Institute of Public Health, Oslo, Norway. Strains were mainly from the years 1997–2006, with about half of the strains isolated prior to 2000, and the other half from 2000 to 2006. Sequence analysis of the *fhbp* gene indicated that 39 strains were from the A subfamily and 61 strains from the B subfamily. These strains were further characterized for fHBP surface expression level. Two *fhbp* gene knockout mutants, one from each subfamily, were generated by insertional inactivation of the chromosomal *fhbp* gene of MnB strains [16]. Available PorA subtype information was provided by the sending reference laboratories. Representatives of prevalent PorA subtypes were included in the collection.

2.2. Recombinant fHBP (rfHBP)

Two variants of fHBP, one from each subfamily (A05 and B01), were recombinantly expressed in *Escherichia coli* as lipoproteins, and highly purified using standard chromatographic methodologies [16].

2.3. Generation of rabbit rfHBP immune sera

Preparation of immune sera was previously described [23]. Briefly, New Zealand White female rabbits, 2.5–3.0 kg, obtained from Charles River Canada (St. Constant, QC, Canada), were prescreened by whole cell ELISA to identify those with low reactivity against two different meningococcal strains (one from each fHBP subfamily). The rabbits, in general, had very low backgrounds, and those with the lowest values were selected for use. The rabbits were vaccinated intramuscularly at weeks 0, 4, and 9 with either monovalent rfHBP-A05, monovalent rfHBP-B01 or a bivalent rfHBP-A05 + B01 vaccine. Each dose contained 100 μg of protein for the monovalent vaccines and 100 μg of each protein for the bivalent vaccine, formulated with 250 μg $AlPO_4$. Pre-immune (week 0) and immune (week 10) serum samples were obtained for analyses. All animal protocols employed in this study adhered to the established Institutional Animal Care and Use Committee guidelines.

2.4. Human bivalent rfHBP immune sera

Immune sera from 5 human volunteers who had received 3 doses of the bivalent rfHBP vaccine as part of a phase 1 safety trial [24] were pooled for evaluating the breadth of bactericidal activity against 45 diverse MnB clinical isolates. The immune sera included in the pool were from individuals who had a greater than 4-fold rise (pre-immune compared to post dose 3) in SBA titers against six divergent MnB strains investigated in the clinical study. Four of the five sera were from individuals in the highest vaccine dosage (200 μg) group, and one was from the intermediate dosage (60 μg) group. A pool of subject matched pre-immune sera was also prepared for baseline SBA analyses.

2.5. Flow cytometry analysis

Surface reactivity and specificity of the rabbit monovalent or bivalent fHBP immune sera was evaluated against 10 MnB strains that expressed diverse fHBP variants (5 from subfamily A and 5 from subfamily B). Two *fhbp* gene knockout mutants, one from each subfamily, were used as controls. Bacteria were grown and prepared as described previously [23]. Bacterial cells were first incubated (30 min at 4 °C) with rabbit pre-immune or immune sera that were diluted 1:50 in phosphate-buffered saline pH 7.0 (PBS) containing 1% bovine serum albumin (BSA/PBS). After washing with the same buffer, cells were stained with FITC-goat anti-rabbit IgG (Cat# 111-095-144, Jackson ImmunoResearch Labs, West Grove, PA, diluted 1:100 in 1% BSA/PBS) for 30 min at 4 °C. Samples were assessed using a BD LSR II flow cytometer, and analyzed using FlowJo v7 software (Treestar, Ashland, Oregon). Mean fluorescence intensity (MFI) in the FITC channel was determined for each sample after gating on bacterial cells in the logarithmic forward scatter (FSC) vs side scatter (SSC) dot plot.

Surface expression of fHBP on a collection of 100 MnB isolates was measured using the monoclonal antibody (mAb), MN86-994-11. This anti-fHBP mAb recognizes a conserved epitope common to both fHBP subfamilies, and was used in flow cytometry following procedures described previously [23].

2.6. Multi-Locus sequence typing

MLST analysis was performed according to the protocols at the MLST website, http://pubmlst.org/neisseria/.

2.7. Serum bactericidal assay

Serum bactericidal antibodies in rabbits or humans immunized with rfHBP vaccine were determined using SBAs with human