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Characterization of *Escherichia coli* K1 colominic acid-specific murine antibodies that are cross-protective against *Neisseria meningitidis* groups B, C, and Y

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ABSTRACT

The capsular polysaccharide (PS) of *Neisseria meningitidis* serogroup B (NMGB) is α(2-8)-linked *N*-acetylneuraminic acid (Neu5Ac), which is almost identical to the *O*-acetylated colominic acid (CA) of *Escherichia coli* K1 Although *E. coli* K1 has long been known to elicit cross-protective antibodies against NMGB, limited information on these highly cross-reactive antibodies is available.

In the present study, six new monoclonal antibodies (mAbs) specific to both *E. coli* K1 CA and NMGB PS were produced by immunizing Balb/c mice with *E. coli* K1, and their serological and molecular properties were characterized, together with 12 previously reported hybridoma mAbs.

Among the bactericidal mAbs against NMGB, both HmenB5 and HmenB18, which are genetically identical though of different mouse origins, were able to kill serogroup C and Y meningococci.

Based on SPR sensograms, the binding affinity of HmenB18 for PS was suggested to be associated with at least two different binding forces: the polyanionicity of Neu5Ac and an interaction with the *O*-acetyl groups of Neu5Ac.

Molecular analysis showed that similar to most mAbs presenting a few restricted V region germline genes, the V region genes of HmenB18 were 979% and 986% identical to the closest IGHV1-1401 and IGLV15-10301 germline gene alleles, respectively, and V-D-J editing in this mAb generated an unusually long VH-CDR3 sequence (17 amino acid residues), containing one basic arginine, two hydrophobic isoleucine residues and a 'YAMDY' motif.

Models of the mAb combining sites demonstrate that most of the mAbs exhibited a wide, shallow groove with a high overall positive charge, as seen in mAb735, which is specific for a polyanionic helical epitope. In contrast, the combining site of HmenB18 was shown to be wide but to present a relatively weak positive charge, consistent with the extensive recognition by HmenB18 of the various structural epitopes formed with the Neu5Ac residue and its *O*-acetylation.

1. Introduction

Neisseria meningitidis is one of the most common causes of bacterial meningitis and sepsis (Rosenstein et al., 2001). Based on the capsular polysaccharide (PS) of *N. meningitidis*, which is one of its major essential virulence factors, the bacterium can be classified into 13 distinct serogroups. Of these 13 serogroups, six (serogroups A, B, C, W-135, X and Y) are responsible for most meningococcal disease around the world (Xie et al., 2013). Because antibodies specific for meningococcal PS are protective against invasive meningococcal diseases (IMD) (Gill et al., 2011; Pace, 2009), meningococcal PS-based vaccines targeting serogroups A, C, W-135, and Y have been developed as a form of meningococcal capsule conjugated to carrier proteins such as tetanus toxoid and CRM_{197} (Pichichero, 2013). However, meningococcal PS-based vaccines for serogroups B and X are still not available.

N. meningitidis serogroup B (NMGB) PS is a homopolymer of α(2-8)-linked *N*-acetylneuraminic acid (Neu5Ac), which is almost

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identical to the *O*-acetylated colominic acid (CA) of *Escherichia coli* K1 (*E. coli* K1 CA), a major cause of neonatal meningitis, urinary tract infections and systemic infections (Glode et al., 1977). α(2-8)-linked Neu5NAc is also found as a long chain of polysialic acid (PSA) linked to the neural cell adhesion molecule (N-CAM) in the human fetal stage (Finne et al., 1983). PSA is recognized as a self-antigen by host immune cells, and this self-recognition results in the low immunogenicity of NMGB PS (Wyle et al., 1972). Despite the low immunogenicity of NMGB PS, the fear of any potential immunopathology caused by autoreactive anti-NMGB PS antibodies has hindered the development of PS-based NMGB vaccines (Edelman, 1983). In an attempt to overcome both the low immunogenicity and potential autoimmunity caused by NMGB PS, various alternative antigens have been introduced (Granoff et al., 1998; Shin et al., 2001). An *N*-propionylated (N-Pr) NMGB PS-conjugated vaccine has been widely studied as an alternative PS antigen for eliciting protective antibodies that are bactericidal against NMGB, but not cross-reactive with human PSA (Flitter et al., 2010; Granoff et al., 1998; Jennings et al., 1987; Moe et al., 2009; Pon et al., 1997).

In a previous study, we produced a panel of 12 hybridoma cell lines expressing mAbs specific for NMGB by immunizing mice with *E. coli* K1 bacteria (Shin et al., 2001). Most of the mAbs were bactericidal against NMGB in vitro, while showing no or decreased cross-reactivity with human PSA expressed on CHP-134 neuroblastoma cells. In particular, it was interesting to find that one mAbs (HmenB5) was cross-reactive with serogroups B, C and Y and killed them through activating rabbit complement. Although NMGB PS is not *O*-acetylated, other cross-reactive bacteria commonly present high numbers of *O*-acetylated Neu5Ac residues within their capsular PSs (Bhattacharjee et al., 1976; Claus et al., 2004; Jann and Jann, 1983; Lemercinier and Jones, 1996; Longworth et al., 2002; Orskov et al., 1979) (Fig. 1). Thus, we hypothesized that the extensive cross-reactivity of HmenB5 with various capsular PSs may be dependent on the degree of *O*-acetylation in the capsular PS. This hypothesis was supported by experimental evidence showing that HmenB5 did not kill meningococcal serogroup W-135, whose capsular PS is less *O*-acetylated (data not shown) (Claus et al., 2004; Trotter et al., 2012).

Anti-PSA antibodies elicited by NMGB PS have been studied through serology and molecular immunology. Among these antibodies, mAb735 is the best characterized in terms of its antigenic specificity and through molecular analyses of its variable (V) region genes, structural crystallography, and three-dimensional modeling (Berry et al., 2005; Frosch et al., 1985; Hayrinen et al., 1989, 2002; Moe et al., 2006; Nagae et al., 2013). Despite of the detailed molecular knowledge of the cross-reactive antibodies elicited by NMGB PS, the molecular properties of the cross-reactive antibodies elicited by *E. coli* K1 CA are not fully understood. Although the development of a PS-based NMGB PS vaccine has long been hindered by a fear of α(2-8)-Neu5Ac as a cause of autoimmune diseases attributed to autoantibodies, this safety issue is controversial due to the lack of clinical evidence of pathology or autoimmune diseases induced by α(2-8)-Neu5Ac antibodies, even in neonates and infant mothers who have recovered from IMD (Robbins et al., 2011). In addition to NMGB and *E. coli* K1, other bacterial species, such as *Pasteurella haemolytica* A2 and *Moraxella nonliquefaciens*, have been found to produce α(2-8)-Neu5Ac as a virulence factor (Devi et al., 1991; Puente-Polledo et al., 1998). Thus, research on the anti-PSA antibodies elicited by bacterial α(2-8)-Neu5Ac must be expanded.

In the present study, we produced six new hybridoma cell lines expressing anti-*E. coli* K1 CA mAbs that are cross-reactive with NMGB PS by immunizing mice with *E. coli* K1 and characterized them through bactericidal assays, surface plasmon resonance analysis, and a V region germline gene analysis using twelve previously described mAbs (Shin et al., 2001). The results were compared to previously reported data on the anti-PSA antibodies elicited by NMGB PS and used for three-dimensional (3D) structural modeling of mAb combining sites to provide insight into the structural differences among the *E. coli* K1 CA-induced antibodies specific for serogroup B, C, and Y meningococci.

2. Materials and methods

2.1. Ethics statement

All animal handling works were performed in accordance with the Korean Food and Drug Administration (KFDA) guidelines. Protocols were reviewed and approved by the animal IRB at Yonsei University (approval No. 10-082-1). All efforts were made to minimize suffering.

2.2. Bacterial strains and reagents

We used various strains of *N. meningitidis* in this study, from serogroups A (M239), B (ATCC13090), C (BB-305), and W-135 (NCCP15745) as well as three Y strains (S-1975, HF13, and HF74) (Park et al., 2005; Shin et al., 2001). HF13 and HF74 were provided by Dr. Carl E. Frasch of CBER, USFDA (Caldwell et al., 2003). All meningococcal strains were grown on chocolate agar plates in a candle jar. The *E. coli* K1 RS218 (serotype O18, K1, H7) and K1 EV11 strains were grown in Luria-Bertani (LB) broth or on LB agar plates. All bacteria were aliquoted in Hank's balanced salt solution (HBSS) containing 20% glycerol and stored at −70 °C before use. Purified capsules of serogroups C and Y and *E. coli* K1 CA were obtained from Merrell-National Laboratories (Cincinnati, OH (lot No.1964)), CBER of the USFDA (Dr. Carl E. Frasch) and the University of Rochester, NY (Dr. W. Vann), respectively. Colominic acid (10 mg) was treated with 0.2N NaOH at 37 °C for 2 h and then neutralized with 6N HCl for de-*O*-acetylation.

2.3. Mouse hybridoma cell lines

Six new hybridoma cell lines expressing mAbs specific for NMGB PS were generated according to a previously described protocol (Shin et al., 2001). Briefly, BALB/cByJ mice from Jackson Laboratory (Bar Harbor, ME) were intraperitoneally immunized with the *E. coli* K1 RS218 strain four times: twice with 1×10^7 CFU on days 0 and 4 and twice with 1×10^8 CFU on days 7 and 10. Their spleens were then harvested on day 13 for fusion with Sp2/0-Ag14 myeloma cells. The hybridoma clones were primarily screened via ELISA with *E. coli* K1 CA, and the cross-reactivity of mAbs with NMGB PS was confirmed through ELISA with heat-killed NMGB (ATCC13090). Of the six new hybridoma clones, five presented immunoglobulin (Ig) M kappa [IgM(κ)] and one presented IgM lambda [IgM(λ)], as shown in Table 1 All mAbs were concentrated with ammonium sulfate, dialyzed in PBS, and fractionated via gel-filtration chromatography with Sephacryl S-300HR (Pharmacia, Uppsala, Sweden) (Shin et al., 2001).

2.4. Complement-mediated bactericidal assay

Bactericidal assays were performed in 96-well microtiter plates (Corning, NY) as described previously (Shin et al., 2001). Briefly, 30 μL of a bacterial suspension containing 2500 CFU, 50 μL of an appropriately diluted antibiotic-free antibody, and 20 μL of baby rabbit complement were added to each well. The concentration of complement was 3–20%, depending on the susceptibility of the bacteria to this complement, and an isotype-matched irrelevant mAb was used as a negative control. Serogroups A, B, C, W-135 and Y were employed as target bacteria. After a 1-h incubation with shaking, 10 μL of the reaction mixture was