Analysis of Human Serum Immunoglobulin G against O-Acetyl-Positive and O-Acetyl-Negative Serogroup W135 Meningococcal Capsular Polysaccharide

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The capsular polysaccharide of Neisseria meningitidis serogroup W135 is expressed in both O-acetyl-positive (OA+) and O-acetyl-negative (OA−) forms. This study investigates the impact of OA status (OA+ versus OA−) on serological measurements of anti-W135 immunoglobulin G (IgG) antibodies in immunized adults. W135-specific serum antibody assignments were made for 28 postimmunization sera from adults by enzyme-linked immunosorbent assay using the meningococcal standard reference serum CDC1992. The established IgG concentration in micrograms per milliliter ([IgG]μg/ml) for CDC1992 against OA+ antigen (16.2 μg/ml) was used as a reference to assign a concentration of 10.13 μg/ml IgG against OA− antigen by cross-standardization. Overall, the IgG assignments for these sera were higher against OA+ antigen (geometric mean concentration [GMC] = 7.16 μg/ml) than against OA− antigen (GMC = 2.84 μg/ml). However, seven sera showed higher specific [IgG]μg/ml values against the OA+ antigen than against the OA− antigen. These sera were also distinguished by the inability of fluid-phase OA− antigen to compete for antibody binding to OA+ solid-phase antigen. Although there was no overall difference in functional activity measured by complement-mediated serum bactericidal assay (SBA) against OA+ and OA− target bacteria (geometric mean titers of 9,642 and 9,045, respectively), three serum specimens showed a large difference in SBA antibody titers against OA+ versus OA− W135 target bacteria, which may reflect different epitope specificities for these sera. Our data indicate that, for some sera, the agreement in anti-OA+ versus anti-OA− W135 IgG assignments is serum specific and does not reflect the functional (killing) activity in vitro.

Neisseria meningitidis is a gram-negative bacterial pathogen that causes sporadic and epidemic disease worldwide (1). Although capsular serogroups A, B, and C are responsible for most reported illnesses, serogroup W135 disease incidence has been on the rise over the past several years (24). The majority of clinical W135 isolates (approximately 90%) have been reported to express O-acetyl-negative (OA−) capsular polysaccharide (20). However, no data that show a relationship between capsular OA status and the severity or outcome of disease have been reported. Currently, there is one licensed vaccine in the United States (Menomune; Aventis) that provides coverage against serogroup W135 as well as serogroups A, C, and Y. Improvements in vaccine technology over the last decade have led to the development and licensure of protein-polysaccharide conjugate vaccines that provide superior protection against serogroup C-related illness in young children, and the future holds promise for a multivalent meningococcal vaccine based on similar technologies (see reference 25 for a review).

Meningococcal capsular antigens are relatively simple carbohydrates that are anchored to the bacterial surface by the lipid moiety of phosphatidic acid (3, 5, 6, 14, 16, 18, 19, 29). The Y and W135 antigens consist of repeating disaccharide units that present few epitopes to the mammalian immune system (5, 14, 16). It has been shown that bactericidal serum antibodies specific for meningococcal capsular polysaccharides (MnPS) are important for protection against meningococcal disease (12). Serogroups Y (6-GlcNAc-α1-4-NeuAcα2) and W135 (6-Galp-α1-4-NeuAcα2) MnPS are structurally related group II capsular polysaccharides with relatively high negative-charge densities. According to a recent study in the United Kingdom, approximately 79% of serogroup Y strains and 8% of serogroup W135 strains express OA-substituted MnPS (20). Substitutions have been observed at positions O-7 and O-9 on the sialic acid residues of Y and W135 MnPS. OA groups have been shown to migrate from O-7 to O-9 during storage of W135 antigen in aqueous solution (16).

Complement-mediated immunoglobulin-dependent serum bactericidal activity has been shown to correlate with protection against meningococcal serogroup C disease (12). Consequently, the World Health Organization Department of Immunization, Vaccines, and Biologicals recommends that vaccine manufacturers use the serum bactericidal assay (SBA) as a potential surrogate for meningococcal vaccine efficacy (32). However, due to variability and sensitivity issues associated with the SBA, the World Health Organization also recommends that the enzyme-linked immunosorbent assay (ELISA)
be used to quantitate capsule-specific humoral immunoglobulin G (IgG).

Published meningococcal ELISA methods employ methylated human serum albumin (mHSA) as a binding agent to promote the adsorption of the anionic polysaccharides to the assay well surface (2, 21). These assays use a standard reference serum, such as CDC1992, to calculate IgG concentrations in micrograms per milliliter [(IgG)μg/ml] in unknown samples. Anti-MnPS immunoglobulin concentrations have been assigned to CDC1992 by cross-standardization in studies reported elsewhere (9, 13, 15). Previously, we showed that for ELISA procedures involving Y and W135 MnPS antigens, the optimal assay plate coating concentrations are serum specific (10), and therefore, interlaboratory agreement may be influenced by antigen coating concentration.

We examined serum IgG in postimmunization human sera against O-acetyl-positive (OA+/−) and OA− W135 capsular polysaccharides by ELISA and against isogenic OA+ and OA− W135 target strains by SBA. Sera were from adults immunized with a licensed meningococcal polysaccharide vaccine (Menomune [serogroup A, C, Y, and W135 vaccine]; Aventis). Note that the vaccine formulation used in this study contains OA− W135 MnPS. The O-acetyl status of the W135 antigen used for serological testing dramatically affected [IgG]μg/ml assignments and bactericidal titers for some individual serum specimens. However, the majority of specimens showed essentially no discrimination between OA+ and OA− W135 targets.

MATERIALS AND METHODS

Materials. (i) Antibody-binding assays. Various purified OA+ and OA− MnPS were obtained from Wyeth (Sanford, NC) and from the National Institute for Biological Standards and Controls (NIBSC, Hertfordshire, United Kingdom). Lots K13-1001, -1002, and -1004 (Wyeth) consist of OA− W135 MnPS. Lots L20265-178 (Wyeth) and 01-428 (NIBSC) consist of OA+ W135 MnPS. Lot 01-429 (NIBSC) consists of OA− Y MnPS. Polysorp medium-binding 96-well microtiter assay plates were purchased from Nalge Nunc (Naperville, IL). The binding agent, mHSA, was obtained from NIBSC or was prepared by Wyeth as previously described (2, 21). The following antigen-coating buffer was used for antigen adsorption to the 96-well microtiter plates: phosphate-buffered saline (PBS) with 0.02% sodium azide (137 mM NaCl, 0.1 mM CaCl2, 2.1 mM KH2PO4, 7 mM Na2HPO4 · 7H2O, and 0.02% NaN3, pH 7). The following antibody dilution buffer was used for generating dilutions of sera and goat anti-human IgG–alkaline phosphatase–conjugated antibody (AP conjugate): PBS (see recipe above) with 5% fetal bovine serum and 0.1% Brij-35, pH 7. AP conjugate was purchased from Sigma-Aldrich (St. Louis, MO). The substrate dilution buffer was composed of 0.5 mM MgCl2, 6H2O in 1 M diethanolamine–HCl solution, pH 9.8. The reaction stop solution was composed of 3 M NaOH. The assay plate wash buffer consisted of Tris-buffered saline with 0.01% Brij-35 (137 mM NaCl, 0.08 mM CaCl2, 4.9 mM CH3NO2, 32.1 mM CH3NO2 · HCl, 2.1 mM KCl, and 0.01% Brij-35, pH 7). Plates were washed on a Bio-Tek model EL-404 or model EL X-405 automated microtiter plate washer (Bio-Tek Instruments, Winooski, VT). Colorimetric detection of developed assay plates was performed in a SpectroMax Plus spectrophotometer with a continuous filter set to 405-nm detection and 600-nm reference wavelengths (Molecular Devices, Sunnyvale, CA). Raw data were collected and processed using validated proprietary software (Wyeth Research, Rochester, NY).

(ii) SBAs. Sterilin 96-well U-bottom plates (Stadfords, United Kingdom) were used in all SBAs. The assay buffer, Hank's balanced salts, was prepared from InviTrogen; 0.5% bovine serum albumin (Sigma) was added to this assay buffer. The exogenous complement source, pooled sera from 3- to 4-week-old rabbits, was purchased from PelFreez (Brown Deer, WI). Bacteria were cultured on Columbia blood agar with 5% defibrinated horse blood (Oxoid, Basingstoke, United Kingdom) for all assays.

RESULTS

CDC1992 cross-standardization. The published meningococcal W135-specific IgG concentration assignments for CDC1992 were generated with OA− W135 MnPS by cross-standardization in the ELISA with previously assigned values (9). The current study extends the cross-standardization tech-
nique to assign a value to CDC1992-specific IgG recognizing OA\(^{-}\) MnPS of W135. All experiments were performed with MnPS and mHSA at 5 µg/ml each in antigen-coating buffer (PBS) (see “Materials” above). Assays performed with OA\(^{-}\) W135 MnPS coating antigens and OA\(^{-}\) W135 reference antigen yielded 79 data points from four experiments. From these data, a value of 10.13 ± 0.59 µg/ml (mean ± standard deviation; coefficient of variation [CV] = 5.8%) of OA\(^{-}\) W135-specific IgG was assigned to CDC1992, and this value was used in subsequent experiments.

To confirm this assignment, cross-standardization experiments were carried out with OA\(^{+}\) Y MnPS reference antigen against OA\(^{-}\) W135 coating antigen, which yielded 74 data points over four experiments. Cross-standardization against OA\(^{+}\) Y MnPS yielded a value of 9.55 ± 0.71 µg/ml (CV = 7.5%, 59 data points) in three experiments run concurrently with experiments described above involving OA\(^{-}\) W135 reference antigen, and a mean value of 10.09 µg/ml ± 0.57 (CV = 5.6% from 15 data points) was yielded in a separate experiment. These values agree with the assignment reported above for the OA\(^{-}\) W135 reference antigen.

**W135 MnPS-specific serum [IgG]µg/ml.** The ELISA method was used to measure concentrations of anti-OA\(^{-}\) and anti-OA\(^{+}\) W135 serum IgG in immunized subjects. All twofold serum dilutions were transferred side-by-side to assay plates coated with either OA\(^{-}\) or OA\(^{+}\) MnPS. Three data points were used to calculate the mean [IgG]µg/ml values for each serum. Overall, the IgG concentrations were determined for all 28 immunized individuals, and the geometric mean concentrations (GMC) against OA\(^{-}\) and OA\(^{+}\) MnPS were calculated to be 7.16 µg/ml and 2.82 µg/ml, respectively. All sera had measurable IgG antibodies to OA\(^{-}\) W135 antigen (Table 1). However, the sera from seven individuals, HMNP01-GG, -JJ, -XX -YY, -HH, -PP, and -ZZ, showed higher [IgG]µg/ml values against OA\(^{-}\) W135 antigen than against OA\(^{+}\) antigen (ratio of >4.0). Four of these seven sera, HMNP01-PP, -JJ, -XX, and -YY, contributed the most to the overall difference between anti-OA\(^{-}\) and -OA\(^{-}\) W135 [IgG]µg/ml assigned values. To illustrate this point, statistical analyses were performed with log-transformed data sets generated from an ELISA using OA\(^{-}\) and OA\(^{+}\) samples. In this context, the overall correlation of the data is low (Pearson correlation = 0.82; concordance correlation = 0.75). However, the correlation improved significantly when serum specimens HMNP01-GG, -JJ, -XX, and -YY were removed from the analysis (Pearson correlation = 0.97; concordance correlation = 0.94). This result is not unexpected, considering the relatively large overall disparity in specific [IgG]µg/ml values between OA\(^{-}\) and OA\(^{+}\) sample assays, especially for specimens HMNP01-GG (difference ratio = 30.6) and HMNP01-JJ (difference ratio = 434.8). The result was confirmed in subsequent ELISA experiments with alternate antigen lots. Note that other serum specimens (e.g., HMNP01-PP and -ZZ) also showed relatively large difference ratios between anti-OA\(^{-}\) and -OA\(^{-}\) W135 [IgG]µg/ml values.

### TABLE 1. Anti-W135 MnPS mean serum IgG concentrations

| Serum          | Anti-W135 IgG (µg/ml) | Difference ratio (OA\(^{-}\)/OA\(^{+}\)) | SBA titer for: | Difference ratio (OA\(^{-}\)/OA\(^{+}\)) |
|----------------|-----------------------|----------------------------------------|----------------|----------------------------------------|
|                | OA\(^{-}\)             | OA\(^{+}\)                              | Strain 2144 (OA\(^{-}\)) | Strain 3149 (OA\(^{+}\)) |
| HMnP01-DD      | 4.47                  | 3.16                                   | 1.4            | 65,536                                 | 131,072                              | 0.5 |
| HMnP01-EF      | 2.12                  | 1.67                                   | 1.3            | 65,536                                 | 4,096                                 | 16.0 |
| HMnP01-FF      | 1.63                  | 0.56                                   | 2.9            | 8,192                                  | 16,384                                | 0.5 |
| HMnP01-GG      | 203.02                | 6.63                                   | 30.6           | 131,072                                | 65,536                                | 2.0 |
| HMnP01-HH      | 0.32                  | 0.05                                   | 6.4            | 1,024                                  | 32,768                                | 0.031 |
| HMnP01-II      | 8.93                  | 7.44                                   | 1.2            | 32,768                                 | 32,768                                | 1.0 |
| HMnP01-JJ      | 34.78                 | 0.08                                   | 434.8          | 384                                    | 8,192                                 | 0.047 |
| HMnP01-KK      | 17.41                 | 13.47                                  | 1.3            | 16,384                                 | 4,096                                 | 4.0 |
| HMnP01-LL      | 39.98                 | 30.93                                  | 1.3            | 262,144                                | 131,072                               | 2.0 |
| HMnP01-MM      | 68.21                 | 43.36                                  | 1.6            | 65,536                                 | 32,768                                | 2.0 |
| HMnP01-NN      | 47.18                 | 29.82                                  | 1.6            | 2,048                                  | 2,048                                 | 1.0 |
| HMnP01-OO      | 0.92                  | 0.56                                   | 1.6            | 65,536                                 | 131,072                               | 0.5 |
| HMnP01-PP      | 1.89                  | 0.41                                   | 4.6            | 4,096                                  | 10,246                                | 4.0 |
| HMnP01-QQ      | 2.14                  | 1.52                                   | 1.4            | 16,384                                 | 8,192                                 | 2.0 |
| HMnP01-RR      | 0.5                   | 0.2                                    | 2.5            | 16,384                                 | 16,384                                | 1.0 |
| HMnP01-SS      | 58.61                 | 40.46                                  | 1.4            | 32,768                                 | 32,768                                | 1.0 |
| HMnP01-TT      | 33.6                  | 30.14                                  | 1.1            | 65,536                                 | 32,768                                | 2.0 |
| HMnP01-UU      | 14.75                 | 12.86                                  | 1.1            | 8,192                                  | 4,096                                 | 2.0 |
| HMnP01-TV      | 0.82                  | 0.21                                   | 3.9            | 2,048                                  | 2,048                                 | 1.0 |
| HMnP01-WW      | 138.38                | 122.44                                 | 1.1            | 8,192                                  | 4,096                                 | 2.0 |
| HMnP01-XX      | 10.46                 | 2.02                                   | 5.2            | 16,384                                 | 8,192                                 | 2.0 |
| HMnP01-YY      | 8.7                   | 0.7                                    | 12.4           | 2,048                                  | 10,246                                | 2.0 |
| HMnP01-ZZ      | 1.42                  | 0.25                                   | 5.7            | 8,192                                  | 8,192                                 | 1.0 |
| HMnP01-AAA     | 48.35                 | 40.06                                  | 1.2            | 4,096                                  | 4,096                                 | 1.0 |
| HMnP01-BBB     | 31.61                 | 23.83                                  | 1.3            | 8,192                                  | 8,192                                 | 1.0 |
| HMnP01-CCC     | 0.05                  | 0.06                                   | 0.8            | 128                                    | 64                                    | 2.0 |
| HMnP01-DDD     | 37.26                 | 34.1                                   | 1.1            | 8,192                                  | 32,768                                | 0.3 |
| HMnP01-EEE     | 0.54                  | 0.83                                   | 0.7            | 512                                    | 1,024                                 | 0.5 |

Overall GM\(^{a}\) 7.16 2.84 9,042 9,045

\(^{a}\) Mean value of three data points (LLD = 0.1 µg/ml).

\(^{b}\) GMC, geometric mean.
However, the values were relatively low compared to those of other sera in the study and did not have a large impact on the overall GMC against OA⁺ versus OA⁻ antigen. Therefore, specimens HMNP01-GG, -JJ, -XX, and -YY were selected for further analysis.

Our previous publication showed that antigen-coating conditions for Y and W135 MnPS impact the serum IgG binding profiles selectively (10). Therefore, we analyzed the IgG binding profiles for serum specimens HMNP01-GG, -JJ, -XX, and -YY and for the reference standard CDC1992 against OA⁺ and OA⁻ antigen, and at a 50-fold dilution against OA⁻ antigen. HMNP01-JJ was tested at a 1,000-fold final dilution against OA⁺ antigen and at a 50-fold dilution (lower limit) against OA⁻ antigen. HMNP01-XX was tested at a 400-fold final dilution against OA⁺ antigen and at a 50-fold dilution against OA⁻ antigen. HMNP01-YY was tested at a 300-fold final dilution against OA⁺ antigen and at a 50-fold dilution against OA⁻ antigen. A405, absorbance at 405 nm.

Impact of soluble competitor on specific [IgG]μg/ml. Competition ELISA experiments with solid-phase antigen and fluid-phase competitor were carried out to analyze the specificity of the serum IgG in specimens HMNP01-GG, -JJ, -XX -YY, -UU, -DD, and -DDD against OA⁺ and OA⁻ antigens. Sera were preabsorbed with various concentrations of fluid-phase Y or W135 MnPS (0 to 30 μg/ml in antibody dilution buffer) and then used in competition ELISAs with OA⁺ and OA⁻ antigen. The results from titration curves generated with CDC1992, each serum specimen shows qualitatively the same titration profile against OA⁺ and OA⁻ antigen, except for HMNP01-JJ, which showed no IgG binding profile against OA⁻ antigen and which had an OA⁻ W135-specific [IgG]μg/ml assigned value that was close to the lower limit of detection (LLD) for the assay. These results suggest that the [IgG]μg/ml differences shown in Table 1 for these four sera against OA⁺ and OA⁻ W135 antigen are not a consequence of the antigen-coating concentrations for each antigen used in the ELISA.
against the isogenic parent OA$^+$ target bacteria than against the isogenic OA$^-$ target, therefore, appears inconsistent with the ELISA results for this serum. SBA activity against another OA$^-$ target strain, M01-240303, confirmed the titer to be 384. Therefore, serum specimen Hmnp01-JJ was tested by competition SBA (22). The results are shown in Table 3. The OA$^-$ MnPS was shown to be effective at reducing the bactericidal titer against the OA$^-$ and OA$^+$ W135 target bacteria. However, the OA$^+$ MnPS was not effective at reducing the titer against either W135 strain. Control experiments carried out with other serum specimens (e.g., Hmnp01-DD, -UU, and -DDD) showed that the OA$^+$ W135 competitor was able to significantly reduce SBA titers (up to a $>100$-fold reduction; data not shown) for serum specimens other than Hmnp01-JJ. Control experiments carried out with serogroup C strain C11 showed no reduction in SBA titer in the presence of a soluble W135 MnPS competitor, as expected (Table 3). These data suggest that the bactericidal activity in serum specimen Hmnp01-JJ is directed at epitopes specific to the OA$^-$ MnPS soluble competitor.

To determine if blocking antibodies were the cause of the discrepancy, we mixed equal volumes of serum specimens Hmnp01-JJ and Hmnp01-II and then measured the SBA activity of the pooled sera against the isogenic target strains. The bactericidal titer was reduced twofold compared to that of serum specimen Hmnp01-II, which would be expected in the absence of Hmnp01-JJ blocking antibodies (data not shown). These data are consistent with the hypothesis that the bactericidal antibodies in serum Hmnp01-JJ recognize epitopes specific for the OA$^-$ MnPS competitor. However, it is important to note that the majority of serum specimens from this study showed essentially no difference in bactericidal activity against the isogenic OA$^+$ and OA$^-$ target strains.

**DISCUSSION**

It is reasonable to speculate that both the presence and the position of OA modifications in MnPS may influence immune responses in humans. Several reports have attempted to describe the potential superiority of certain vaccine formulations over others in this regard (2, 4, 11, 23, 27, 28). For example, a recent publication concluded that O-acetyl substitution is critical to the immunogenicity of serogroup A MnPS (4). That report showed higher bactericidal titers (SBA; the serogroup A target strain was OA$^+$ F8238) and higher OA$^+$-specific IgG concentrations (ELISA; the solid-phase antigen was OA$^+$ serogroup A MnPS) in pooled sera from mice immunized with protein-conjugated OA$^+$ serogroup A MnPS than in sera from

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**TABLE 2. Percent competition for selected sera at 30 μg/ml of fluid-phase competitor**

| Serum        | W135 OA$^+$ coating Ag | W135 OA$^-$ coating Ag |
|--------------|-------------------------|-------------------------|
| OA$^+$ W135  | OA$^+$ W135             | OA$^+$ Y$^+$            |
| Hmnp01-Gg    | 93                      | 0                       | 77 87 0                     |
| Hmnp01-Jj    | 92                      | 53                      | ND ND ND                    |
| Hmnp01-Xx    | 90                      | 22                      | 80 85 0                     |
| Hmnp01-Yy    | 93                      | 17                      | 75 75 18                    |
| Hmnp01-Ddd   | 90                      | 18                      | 87 90 0                     |
| Hmnp01-Uu    | 88                      | 73                      | 87 89 0                     |
| Hmnp01-DD    | 86                      | 68                      | 89 87 0                     |

$^a$ Percent competition based on [IgG] μg/ml assignments. ND, not determined.

$^b$ Competitor (30 μg/ml) in antibody dilution buffer. Ag, antigen.

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**TABLE 3. SBA titer for serum specimen Hmnp01-JJ with fluid-phase W135 MnPS competitor**

| Competitor$^a$ | SBA titer for indicated target strain |
|----------------|--------------------------------------|
| OA$^+$ W135 MnPs | 2144 (OA$^+$) 3149 (OA$^-$) C11$^b$ |
| OA$^+$ W135 MnPs | 256–512 8,192 1,024                  |
| OA$^+$ W135 MnPs | 4–8 8–16 1,024–2,048                 |
| None            | 256–512 8,192 512–1,024              |

$^a$ Heterologous serogroup C control target strain.

$^b$ Fluid-phase competitor (200 μg/ml).
mice immunized with chemically de-O-acetylated antigen. However, it was also noted that chemical de-O-acetylation removes the lipid tail associated with the polysaccharide. Therefore, no serology was possible with solid-phase OA- antigen due to poor adsorption of chemically de-O-acetylated MnPS to assay plates. Instead, fluid-phase de-O-acetylated antigen was used in competition assays to measure anti-OA- serogroup A antibodies.

If humoral responses to MnPS are epitope selective, then immunization with highly OA- substituted MnPS may inhibit responses to underlying or adjacent epitopes. This idea was introduced for serogroup C MnPS formulations in a previous report (23), although it should be noted that prior studies found no statistically significant difference in human IgG responses to polysaccharide vaccine formulations containing either OA+ or OA- serogroup C MnPS (2, 27). Clearly, the presence of OA groups introduces unique epitopes to OA+ MnPS and may mask epitopes common to both OA+ and OA- antigens. Therefore, it is also reasonable to speculate that some individuals may respond very well to OA+ specific epitopes and disproportionately poorly to structures common to both antigens that may be blocked by OA substitutions.

Our data do not refute this speculation. Serologic analyses by ELISA show that although the overall serum GMC was higher against OA+ W135 target antigen (see Results), 7 of the 28 volunteers had serum IgG concentrations that were dramatically lower against OA- W135 antigen. For four of these individuals, the overall GMCs against OA+ and OA- W135 antigens were 28.3 μg/ml and 0.85 μg/ml, respectively. Immunization with a formulation containing OA- MnPS may have influenced the ability of these individuals to respond to structures common to OA- and OA+ antigens.

The present study was carried out to determine whether there is a linear association between OA+ and OA- W135 antigen and the outcome of serological testing. The overall correlation between OA- and OA+ IgG[μg/ml] assigned values was low (Pearson correlation = 0.82) for the data described here. Excluding specimens HMnP01-GG, -JJ, -XX, and -YY from the analysis improved the correlation (Pearson correlation = 0.97), suggesting that one or more of these data points is a statistical outlier. An analysis of Studentized residuals showed that the data point representing specimen HMnP01-JJ is the only data point that had a value greater than 3 standard deviations from the mean and, therefore, is an outlier that makes the regression model unstable. The simple linear regression model is not adequate to predict the data point for HMnP01-JJ. The Cook’s D statistic (26) confirmed that this is an influential data point in the overall model, making the regression model unstable. Excluding this data point from the analysis results in an improved model (Pearson correlation = 0.92), which gives a higher estimate of the correlation between these methods.

Our analysis is complicated by the fact that the Menomune formulation contains both OA+ Y and OA+ W135 MnPS. Competition ELISA data show, not surprisingly, that Y and W135 cross-reactive antibodies influence specific IgG measurements. The fact that these cross-reactive components are serum dependent strengthens the argument that individual responses to these antigens in humans are heterogeneous and epitope selective. It will be difficult, if not impossible, to sort out the individual responses to OA+ and OA- W135 antigens in the absence of studies of monovalent MnPS vaccine formulations in humans. Likewise, relatively small amounts of MnPS-specific IgM can have a high bactericidal activity and may have a dramatic impact on the magnitude of SBA titers (30). Although we were not able to measure anti-W135 specific IgM at any antigen-coating concentration (data not shown), the influence of trace amounts of IgM cannot be ruled out as the cause of the discrepancy between ELISA and SBA results for specimen HMnP01-JJ. Ultimately, the gold standard SBA may be more useful for assessing meningococcal vaccine efficacy, given the limitations described here and elsewhere for the ELISA.

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