

Serologic Studies on Group C Meningococcal Vaccines
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Clinical trials have been conducted in the UK on a group C meningococcal polysaccharide (GCMP) conjugate vaccine developed and manufactured at North American Vaccine (NAVA). The trials being evaluated by the Institute of Child Health and Public Health Laboratory Service include adults (1, 2), infants (3), toddlers (4), pre-schoolers, and school-leavers. Serologic studies in this report have been conducted by NAVA on sera from Phase I (adults, 1 IM injection) and Phase II (infants, 3 IM injections, 2-3-4 months) trials. This vaccine consisted of the de-*O*-acetylated form of the polysaccharide (PS) coupled to tetanus toxoid (TT) by reductive amination (5). Each dose contained 10 µg PS, 15.5 µg TT, 0.5 mg aluminum hydroxide, and 0.01% thimerosal. Postimmune sera were sampled at 1 month after each injection.

The de-*O*-acetylated form of GCMP was chosen for its greater immunogenicity as a conjugate when compared with its *O*-acetylated form in mice, which included higher bactericidal (BC) titers (6). Others had previously reported similar results in humans with PS vaccines (7, 8, 9), and more recently with conjugate vaccines in a Phase II toddler study (4). In addition, we have now shown that the de-*O*-acetylated PS can competitively inhibit the serum bactericidal activity (SBA) of NAVA vaccine-induced human antibodies directed against the *O*-acetylated C11 strain at concentrations that are 10-fold lower than that required for the *O*-acetylated PS.

The PS-specific IgG and IgM were measured by ELISA (10), using the CDC 1992 reference serum (11) to standardize the IgG concentrations. For the coat antigen, de-*O*-acetylated GCMP was conjugated by reductive amination to human serum albumin (HSA) as an irrelevant carrier protein that would enable binding to the microtiter plate (10). These HSA conjugates were chosen as coat antigens for their ease of use, stability, and consistency.

Since SBA had previously been correlated with protection using human complement (12, 13), the SBA was determined using adult human sera, as well as infant rabbit sera, for exogenous complement sources in order to assess the functional activity of the antibodies towards the C11 reference strain. The BC titer was defined as the reciprocal serum dilution that resulted in 50% killing (10).

Rabbit complement resulted in BC titers that averaged 4.4-fold higher than what was seen with human complement when assaying infant sera after 1, 2, or 3 injections with the GCMP-TT conjugate vaccine; all but 1 were \leq 12-fold higher (80/81). A strong correlation ($r = 0.80$, $n = 81$), however, was shown for the SBA when comparing rabbit with human complement. All subsequent SBA correlations reported here were performed with rabbit complement.

Strong correlations were seen between the IgG concentration and the BC titer for adults after 1 injection ($r = 0.86$, $n = 30$) and infants ($n = 36$) after 1 ($r = 0.73$), 2 ($r = 0.76$), and 3 ($r = 0.72$) injections. These correlations were well maintained even with inclusion of preimmune sera (e.g., adults: $r = 0.89$). Interestingly, the scatter plots of infant sera at 4 and 5 months (post 2nd and 3rd injections, respectively) were essentially superimposable on that of the adult sera; i.e., the graphic display of SBA per IgG was nearly identical for adults and infants ($r = 0.83$). However, there was a shift in response for the sera obtained from the 1st injection in infants toward lower BC titers per IgG compared to adults. No significant correlation was observed for IgM with SBA in the adults ($r = 0.10$). The infants, however, did show some correlation after the 1st injection ($r = 0.58$) that became weaker by the 3rd injection ($r = 0.33$).

Sera evaluated from 28 UK high school students one month after receiving a licensed meningococcal AC polysaccharide vaccine (ACVAX, SmithKline Beecham, UK) showed strong correlation between IgG and SBA ($r = 0.89$). This scatter plot had also fit the same correlation line as that of the adults receiving the NAVA conjugate vaccine ($r = 0.87$ when combined), although the IgG

and SBA were both significantly reduced for the PS vaccine when compared to the conjugate vaccine ($p < 0.001$).

In summary, strong correlations were routinely observed between IgG and SBA using rabbit complement for both adult and infant human sera. While assays with human complement did result in 4.4-fold lower BC titers compared to rabbit complement, there appeared to be no advantage or necessity to choose human complement over rabbit complement when using the SBA as a serologic surrogate for efficacy.

References

1. Richmond P, Goldblatt D, Fusco PC, Fusco JDS, Heron I, Michon F. 1997. Phase I evaluation of a meningococcal C-tetanus toxoid conjugate vaccine. Abstract G-111, p. 212, American Society for Microbiology, 37th ICAAC (Toronto, Ontario, Canada, 1997), Washington, D.C.
2. Richmond P, Goldblatt D, Heron I, Fusco JDS, Fusco PC, Michon F. 1998. Quantitative and qualitative evaluation of a new meningococcal C conjugate vaccine. Abstract PS 8, p. 10, Abstract Book of the 16th Annual Meeting of the European Society for Paediatric Infectious Diseases (ESPID); Bled, Slovenia; May 27-29, 1998.
3. Richmond P, Borrow R, Clark S, Findlow J, Morris R, and Cartwright K. 1999. Meningococcal C-tetanus toxoid conjugate vaccine is immunogenic and induces memory in infants. Abstract O-13, p. 29, Abstract Book of the 17th Annual Meeting of the European Society for Paediatric Infectious Diseases (ESPID); Heraklion, Crete, Greece; May 19-21, 1999.
4. Richmond P, Cartwright K, Borrow R, Morris R, Clark S, Burrage M, Thornton C, Kaczmarek E, Miller E. 1998. An investigation of the immunogenicity and reactogenicity of three meningococcal serogroup C conjugate vaccines administered as a single dose in UK toddlers. p. 153. In X. Nassif, M.-J. Quentin-Millet, & M.-K. Taha (eds.), Abstracts of the Eleventh International Pathogenic Neisseria Conference (Neisseria '98, Nice, France, Nov. 1-6, 1998).
5. Jennings HJ, Lugowski C. 1981. Immunochemistry of groups A, B, and C meningococcal polysaccharide-tetanus toxoid conjugates. *J. Immunol.* 127:1011-1018.
6. Hronowski L, Di J, Pullen J, Rohrbaugh J, Huang C-H, Michon F, Mates S, Tai JY. 1993. Structure activity studies on *Neisseria meningitidis* group C polysaccharide-protein conjugate vaccines - the effect of O-acetylation on the nature of the antibody response. Abstract E-75, p.155, American Society for Microbiology, 93rd General Meeting (Atlanta, GA, 1993), Washington, D.C.
7. Glode MP, Lewin EB, Sutton A, Le CT, Gotschlich EC, Robbins JB. 1979. Comparative immunogenicity of vaccines prepared from capsular polysaccharides of group C *Neisseria meningitidis* O-acetyl positive and O-acetyl negative variants and *Escherichia coli* K92 in adult volunteers. *J. Infect. Dis.* 139:52-59
8. Steinoff MC, Lewin EB, Gotschlich EC, Robbins JB, Panorama Pediatric Group. 1981. Group C *Neisseria meningitidis* variant polysaccharide vaccines in children. *Infect. Immun.* 34:144-146
9. Peltola HA, Safary A, Kayhty H, Karanko V, Andre FE. 1985. Evaluation of two tetravalent (ACYW₁₃₅) meningococcal vaccines in infants and small children: a clinical study comparing immunogenicity of O-acetyl negative and O-acetyl positive serogroup C polysaccharides. *Pediatrics* 76:91-96
10. Fusco PC, Michon F, Tai JY, Blake MS. 1997. Preclinical evaluation of a novel group B meningococcal conjugate vaccine that elicits bactericidal activity in both mice and nonhuman primates. *J. Infect. Dis.* 175:364-372.
11. Holder PK, Maslanka SE, Pais LB, Dykes J, Plikaytis BD, Carlone GM. 1995. Assignment of *Neisseria meningitidis* serogroup A and C class-specific anticapsular antibody concentrations to the new standard reference serum CDC 1992. *Clin. Diagn. Lab. Immunol.* 2:132-137.
12. Goldschneider I, Gotschlich EC, Artenstein MS. 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. *J. Exp. Med.* 129:1307-1326.
13. Goldschneider I, Gotschlich EC, Artenstein MS. 1969. Human immunity to the meningococcus. II. Development of natural immunity. *J. Exp. Med.* 129:1327-1348.