JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1980, p. 256-263 Vol. 12, No. 2
0095-1137/80/08-0256/08$02.00/0

Comparison of Passive Hemagglutination, Bactericidal Activity, and Radioimmunological Methods in Measuring Antibody Responses to *Neisseria meningitidis* Group A Capsular Polysaccharide Vaccine

HELENA KÄYHTY
Central Public Health Laboratory, Helsinki, Finland

Passive hemagglutination (HA), a bactericidal activity test (BCA), and radioimmunoassay (RIA) were compared in measuring serum antibodies before and after group A meningococcal capsular polysaccharide vaccination of servicemen. The three methods were found satisfactory in demonstrating a response to vaccination in this age group. Of the postimmunization sera, 5% remained without HA and 1% remained without BCA activity; 1% of the postimmunization sera had less than 2 μg of antibody per ml as measured by RIA. Approximately 60% of the serum pairs showed a ≥32-fold rise in HA titer, a ≥25-fold rise in BCA titer, or a ≥4-fold rise in antibody concentration by RIA. A difference in response to two different vaccine lots was seen with RIA and BCA. Although the calculated correlation between the three methods was good, some individual sera gave discrepant results. These could be shown to be due mainly to one of the following factors: low HA titer was due to lack of the immunoglobulin M and A classes of antibodies, low BCA titer was due to the blocking effect of high immunoglobulin A content, and high BCA titer was due to antibodies directed to bacterial components other than the capsular polysaccharide.

About 10 years ago, Gotschlich and his coworkers isolated the capsular polysaccharide from group A and C meningococci (15) and demonstrated that the majority of the bactericidal activity in the sera of patients after meningococcal infection was directed against this component (13). The isolated polysaccharides were immunogenic (14), and their protective efficacy in field trials was promising (4, 10). Since then, vaccines of group A or C meningococcal capsular polysaccharides have been used with success in large vaccination trials as well as to combat epidemics in Africa (8, 30, 31), Finland (22, 23, 25, 26), and Brazil (28, 33). Many different serological methods have been used to measure the antibody response to these vaccines: passive hemagglutination (HA; 3, 15), latex agglutination test (29), immunofluorescent assay (3, 12), opsonizing activity test (27), bactericidal activity test (BCA; 11, 12, 20), and radioimmunoassay (RIA; 7, 17, 23). Some of these methods have been compared in previous studies by Anderson et al. (1), Brandt et al. (5, 6), and Wong et al. (32). These authors have observed some discrepancies between the methods, but have not studied the basis for these discrepancies in detail.

The presence of bactericidal antibodies seems to be correlated with protection from meningococcal infection. Of 54 recruits who fell ill with meningococcal disease, 51 did not have detectable bactericidal antibodies against the causative agent of the disease when their sera, taken a few weeks before disease (12), were assayed. Young children, among whom meningococcal disease is the most common, do not have bactericidal antibodies against meningococci, whereas adults, among whom meningococcal disease is rare, usually have such antibodies (12). Such an adult population has a mean level of anti-meningococcal group A capsular polysaccharide antibodies (anti-MenA) of about 2 μg/ml measured with RIA (23), and thus this level has been considered likely to be protective (21, 23).

The purpose of this study was to correlate group A meningococcal antibodies measured with the three most commonly used methods, HA, BCA, and RIA, using sera collected before and after vaccination. Two different lots of group A meningococcal polysaccharide vaccine were used, and the responses to them are also compared.

MATERIALS AND METHODS

Vaccinations. Military recruits were vaccinated as described earlier (22). Two lots of group A meningococcal capsular polysaccharide vaccine were donated by Merck Sharp & Dohme Research Laboratories, West Point, Pa.). The lot used in February was no.

453, purified by chloroform-butanol extraction (the Sevag method; 15); its molecular weight was given as over 200,000 measured by Sephadex G-200 chromatography. Unfortunately, no K_d data are available, and the material was completely exhausted in the vaccination project. The lot used in June was no. 553 purified by phenol extraction (16); its molecular weight was given as 800,000, and its K_d, measured by Sepharose 4B chromatography, was 0.49 (32). The lyophilized vaccines were kept at −20°C until used and rehydrated when needed. The dose was 50 μg (0.5 ml) given subcutaneously.

Pre- and postimmunization blood samples. Blood samples (5 ml) were taken immediately before and 1 month after vaccination. All sera were stored at −20°C until used. The pre- and postvaccination sera of each individual were tested simultaneously, and a reference serum was included in the set assayed each day.

Serological tests. The HA test was performed as described earlier (15). Fresh human O RH-negative erythrocytes were sensitized with group A polysaccharide lot S719-15P received from Merck Sharp & Dohme.

The BCA test was performed as described earlier (11, 12) using *Neisseria meningitidis* group A strain A1a (from Merck Sharp & Dohme) and fresh absorbed rabbit serum as complement. The highest serum dilution that gave 50% killing was taken as the titer endpoint.

RIA was performed as described earlier (17, 23). In short, 50 μl of serum or its dilution and 50 μl of the labeled MenA antigen (0.1 μg/ml) were mixed in a test tube and kept overnight at 4°C. Then the antigen-antibody complexes were precipitated by ammonium sulfate and counted. The tyramine derivative of group A meningococcal polysaccharide, used for preparing the ^{125}I-labeled MenA, was received from E. C. Gotschlich (Rockefeller University, New York, N.Y.). The preimmunization sera were measured undiluted and diluted 1:5, and the postimmunization sera were measured undiluted and diluted 1:10 in fetal calf serum (Microbiological Associates, Walkersville, Md.). Dilutions of serum ECG (17), received from Gotschlich, were used as standards.

Absorption of sera with MenA polysaccharide was done in some experiments as described (13).

Immunoglobulin class determinations were done by RIA using anti-immunoglobulin G (IgG), -IgM, and -IgA antisera (Dakopatts, Copenhagen, Denmark) instead of ammonium sulfate for precipitation of the antigen-antibody complexes. The assays were performed as follows: 50 μl of the ^{125}I-labeled MenA antigen (0.1 μg/ml) and 20 μl of undiluted serum were mixed and incubated overnight at 4°C. Next day the antibodies and the labeled antigen attached to them were precipitated by adding 300, 70, or 100 μl of anti-IgG, -IgM, or -IgA antiserum, respectively. These amounts were adopted on the basis of data given by the manufacturer and preliminary tests with patient and normal human sera to check completeness of precipitation of antibody. The binding of antigen was linearly correlated with the concentration of immunoglobulins (dilutions of a serum) in the area from 65 to 5%. Sera J and K in Table 4 illustrate typical behavior of pre- and postimmune sera in this assay.

Statistical methods. Geometric mean titers and correlation coefficients were calculated and χ^2 and t-tests were performed using a SR-52 calculator and its Statistics Library from Texas Instruments (Texas Instruments Inc., Dallas, Tex.).

RESULTS AND DISCUSSION

Sensitivity of the three methods. The pre- and postimmunization antibody levels measured by the three methods (Tables 1 to 3) show that the sensitivity of the HA method was the lowest. A total of 87% of the prevaccination sera were negative by HA, compared with only 30% negative by the BCA. By RIA, 39% of the prevaccination sera were below 2 μg/ml, the proposed protective level. However, much lower values could be measured accurately by this method, an advantage when examining sera of infants and children. None of the adult sera in the present material had less than 0.5 μg of anti-MenA per ml.

A response to vaccination was detected by all three methods. Of the postvaccination sera, 5% remained negative by HA, 1% were negative by

TABLE 1. *Anti-MenA measured by HA*

Reciprocal of serum dilution	Prevaccination sera[a] (671)	Postvaccination sera[a]	
		Vaccine 453 (313)	Vaccine 553 (310)
<3.2	**86.6**[b]	4.8	5.2
3.2	9.7	7.4	11.0
10	2.7	21.4	23.8
32	0.7	**30.0**	**31.3**
100	0	23.3	18.4
320	0.3	5.8	7.1
1,000	0	5.4	3.2
≥3,200	0	1.9	0

[a] Percentage of sera in each class. Parentheses indicate number of sera.

[b] Median values are printed in boldface.

TABLE 2. *BCA titers against group A meningococci*

Reciprocal of serum dilution	Prevaccination sera[a] (213)	Postvaccination sera[a]	
		Vaccine 453 (100)	Vaccine 553 (100)
<10	29.1	1	1
10	**37.6**[b]	4	4
50	27.7	30	16
250	4.7	**55**	**44**
1,250	0.9	9	33
6,250	0	1	2

[a] Percentage of sera in each class. Parentheses indicate number of sera.

[b] Median values are printed in boldface.

TABLE 3. *Anti-MenA measured by RIA*

Antibody (μg/ml)	Prevaccination sera[a] (98)	Postvaccination sera[a]	
		Vaccine 453 (43)	Vaccine 553 (47)
0.50[b]–0.99	16.3	0	0
1.00–1.99	22.4	2.3	0
2.00–3.99	**25.5**[c]	11.6	4.3
4.00–7.99	21.4	25.6	14.9
8.00–15.99	10.2	**16.3**	17.0
16.00–31.99	2.1	18.6	**21.3**
32.00–63.99	2.1	16.3	14.9
≥64.00	0	9.3	27.6

[a] Percentage of sera in each class. Parentheses indicate number of sera.
[b] None of the sera was <0.50 μg/ml.
[c] Median values are printed in boldface.

BCA, and 1% were below 2 μg/ml by RIA. The RIA method showed a clear difference between the two vaccine lots used: the geometric mean anti-MenA titer reached after lot 553 was 23.9 μg/ml, but that after lot 453 was only 12.9 μg/ml (Table 3). The BCA titers reached after lot 553 were also somewhat higher than after lot 453 (Table 2), whereas there was no indication of such a lot difference when antibodies were measured by HA (Table 1). Similar findings were made by Brandt et al. (6) comparing HA and RIA. The difference in vaccine response correlated in an expected (6, 19) way with the molecular weight of the polysaccharide of the vaccines. Furthermore, our long-term follow-up data show that persistence of elevated antibody levels correlates significantly with the level of response measured by RIA 3 weeks after vaccination (H. Käyhty, V. Karanko, H. Peltola, S. Sarna, and P. H. Mäkelä, submitted for publication). Accurate determination of the response, as best accomplished by RIA, would therefore seem important for assessing vaccine quality.

The rise in antibody titers in each pair of sera is analyzed in Fig. 1 to 3. No rise in HA or BCA titer was seen in less than 10% of them. A less than twofold rise in antibody concentration measured by RIA was seen in about 15% of the serum pairs. These were usually pairs with a high antibody level already in the prevaccination sample. Approximately 60% of the pairs showed a ≥32-fold rise in HA titer, a ≥25-fold rise in BCA titer, or a ≥4-fold rise in antibody concentration by RIA. Also by this comparison, the two vaccine lots differed most clearly when antibodies were measured by RIA.

It is thus clear that all three methods can be used to measure both the prevaccination antibody levels and the response to vaccination with the current capsular polysaccharide vaccines in adult populations. However, HA, as the least sensitive, has in fact been shown too insensitive to detect vaccine responses in infants in a satisfactory manner (17, 24). For these subjects the most sensitive method, RIA, is definitely to be recommended.

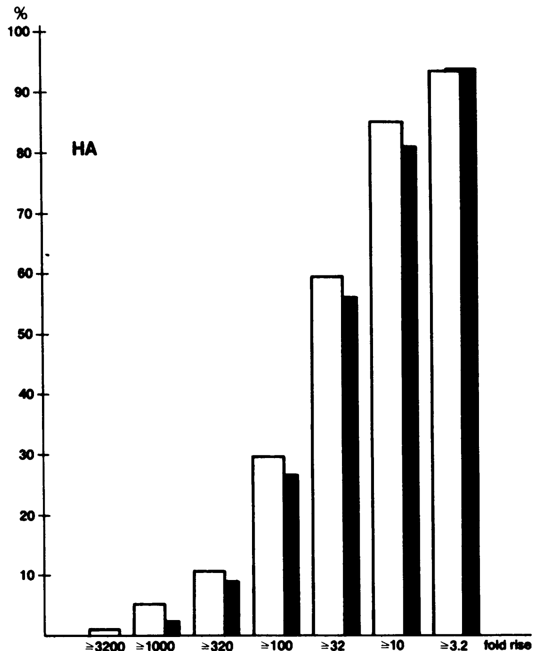

FIG. 1. *Rise in HA titer in paired sera of recruits taken before and 1 month after vaccination with capsular polysaccharide of N. meningitidis group A (open columns, vaccine lot 453; solid columns, vaccine lot 553).*

Correlation between HA and RIA. A priori, HA and RIA could be expected to show good correlation, because they both measure antibodies to the capsular polysaccharide only. However, HA detects mainly IgM (2, 3), whereas the RIA, in which the antibody bound to antigen is precipitated with ammonium sulfate, measures all immunoglobulin classes. The poor sensitivity of the HA was seen in sera with low (<2 μg/ml) anti-MenA values: all these sera were negative or only weakly positive by HA. When these sera were omitted from the calculation, the correlation between HA and RIA was good ($r = 0.70$, $P < 0.001$), and the regression line was linear (Fig. 4).

The analysis of the immunoglobulin class distribution of the anti-MenA (Table 4) shows that a large part of the scatter in HA values at any given level of anti-MenA measured by RIA can be explained by varying immunoglobulin class distribution. This has previously been suggested as a possibility by Brandt and Artenstein (5).

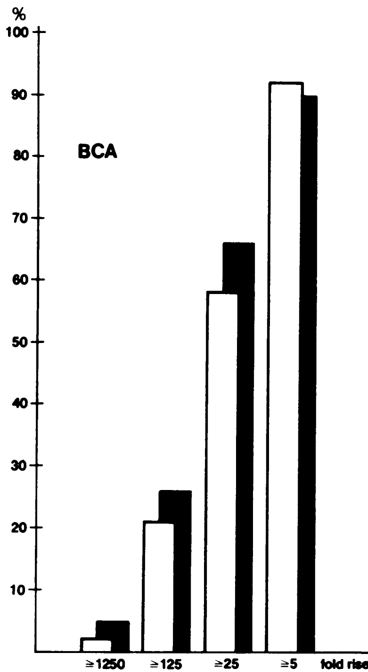

FIG. 2. *Rise in bactericidal antibody titer in paired sera of recruits taken before and 1 month after vaccination with capsular polysaccharide of N. meningitidis group A (open columns, vaccine lot 453; solid columns, vaccine lot 553).*

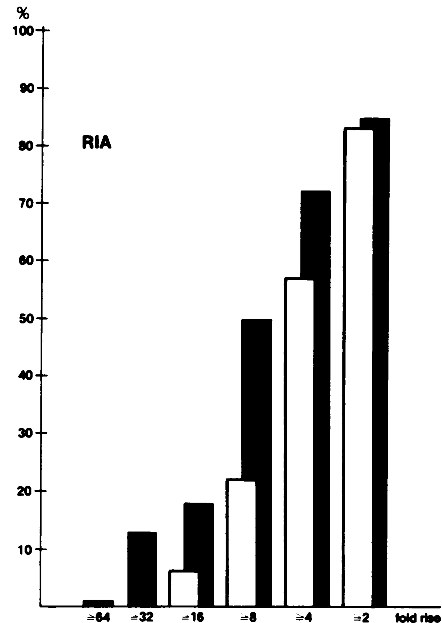

FIG. 3. *Rise in antibody levels measured by RIA in paired sera of recruits taken before and 1 month after vaccination with capsular polysaccharide of N. meningitidis group A (open columns, vaccine lot 453; solid columns, vaccine lot 553).*

For example, sera A to D, which had low HA activity, contained almost exclusively IgG-class anti-MenA. The lack of IgM and IgA anti-MenA in these sera is not unreasonable: all of them were preimmune sera in which the antigenic stimulus may have been a long time ago so that only IgG antibodies would survive.

Correlation between BCA and the other methods. The bactericidal antibody assay differs from both HA and RIA by measuring antibodies not only to the capsular polysaccharide but to other components of the cell wall as well. Specifically, antibodies directed to the major outer membrane protein, the so called serotype antigen, have been shown to be bactericidal (9). Furthermore, the BCA preferentially measures antibodies of the IgG class (2, 14) and has been shown to be inhibited by antibodies of the IgA class (18). The correlation of BCA to HA (Fig. 5) was, however, very good ($r = 0.73$, $P < 0.001$), and that of BCA to RIA (Fig. 6) was good ($r = 0.57$, $P < 0.001$), with linear regression lines when sera with low amounts of antibody (<2 μg/ml by RIA) were omitted from the calculations.

Of 114 sera with measurable HA antibody, almost all (110) also had bactericidal activity, whereas of the 77 HA-negative sera 49 were positive in BCA with titers ranging from 1:10 to 1:250. This may reflect the lower sensitivity of the HA compared to the BCA method, but at least two other factors also play a role. Almost all (47 of 49) of these HA-negative BCA-positive sera were taken before immunization. Half (23) of these 47 contained appreciable amounts of anti-MenA as measured by RIA (ranging from 2 to as high as 26.8 μg/ml). Four of these (A to D) were analyzed as to the immunoglobulin class distribution of the anti-MenA (Table 4). All four had a very low concentration of IgM anti-MenA, explaining the lack of HA activity. The remaining half (24) had less than 2 μg of anti-MenA polysaccharide antibody per ml, consistent with lack of HA activity. In these sera the bactericidal activity is assumed to be directed to components other than the capsular polysaccharide. Antibodies to nonpolysaccharide components could likewise account for the very high BCA titer in some sera with only moderate RIA and HA values. Two such sera, H and I, were further analyzed (Table 4): the distribution of the different immunoglobulin classes of anti-MenA activity was unremarkable. The addition of purified MenA to the sera left a considerable fraction of their bactericidal titer uninhibited, as if indeed due to other than anti-MenA polysaccharide antibodies.

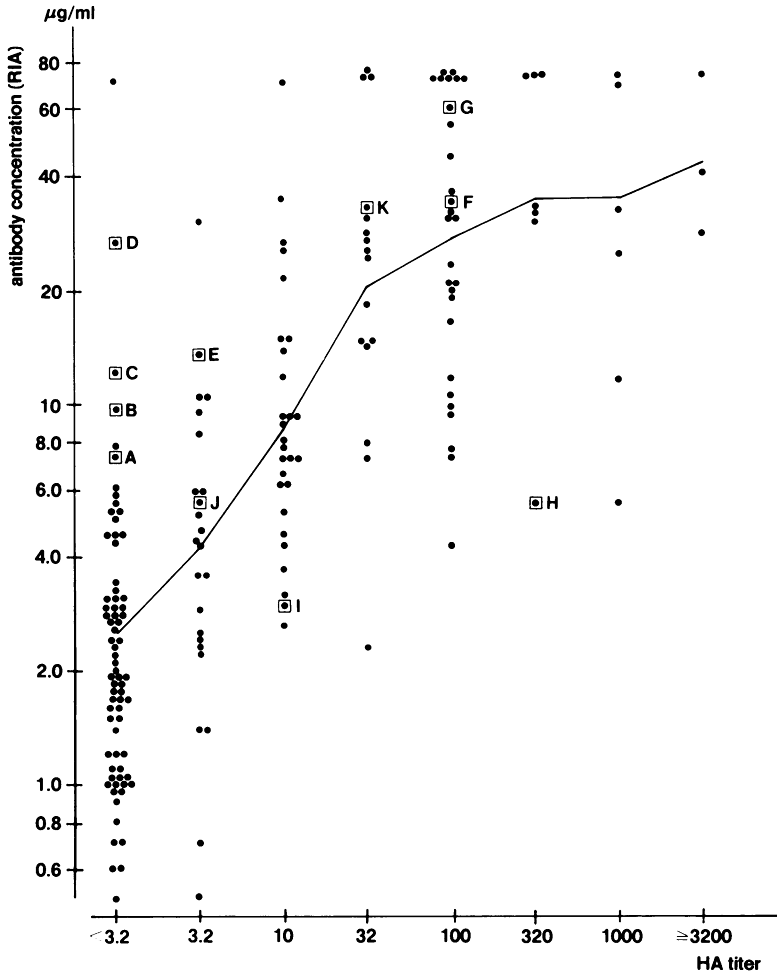

FIG. 4. *Correlation of HA activity and antibody concentration (by RIA) in paired sera of recruits taken before and 1 month after vaccination with capsular polysaccharide of N. meningitidis group A. Sera marked with capital letters A to K are examined in detail in Table 4.*

TABLE 4. *Immunoglobulin classes of the anti-MenA in some sera[a] in which the HA, BCA, and RIA do not agree, and in a normal serum pair (J, K)*

Serum	Immunization	HA titer	BCA titer	RIA (μg/ml)	Antigen binding[b] of:		
					IgG	IgM	IgA
A	Preimmunization	<3.2	50	7.0	10.7	<5.0	<5.0
B	Preimmunization	<3.2	50	9.7	13.3	<5.0	<5.0
C	Preimmunization	<3.2	50	12.1	21.7	6.7	<5.0
D	Preimmunization	<3.2	50	26.8	28.1	~5.0	~5.0
E	Preimmunization	3.2	250	13.7	12.0	5.8	~5.0
F	Postimmunization	100	10	34.6	>65.0	>65.0	45.5
G	Postimmunization	100	10	60.0	>65.0	>65.0	>65.0
H	Postimmunization	320	≥6,250[c]	5.5	47.2	24.2	37.6
I	Postimmunization	10	1,250[c]	3.0	31.7	18.5	46.0
J	Preimmunization	3.2	<10	5.6	5.1	10.0	~5.0
K	Postimmunization	32	250	35.0	44.2	23.1	27.2

[a] Sera A to I, Fig. 4 to 6.
[b] Percentage of ^{125}I-labeled MenA polysaccharide bound.
[c] Titer was 250 after preabsorption with MenA.

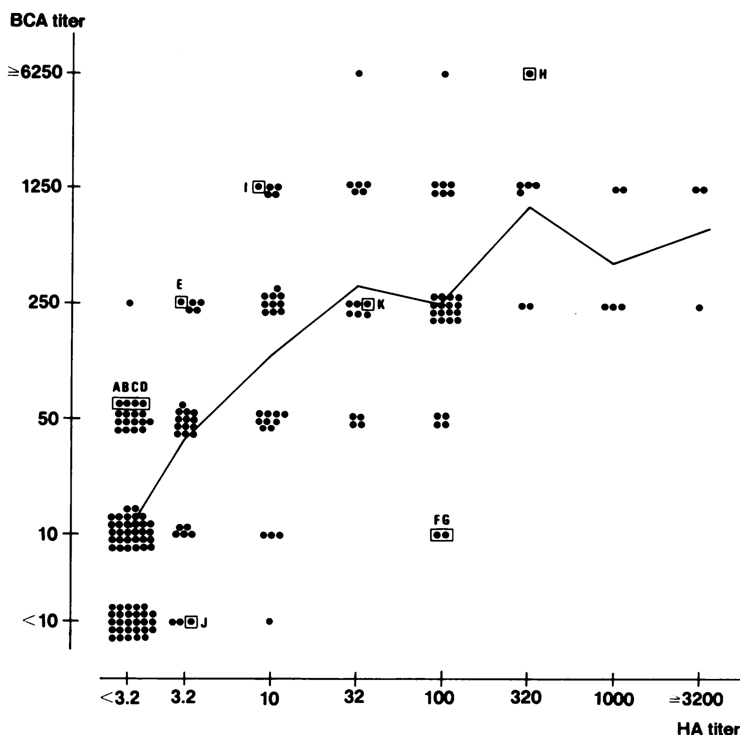

FIG. 5. *Correlation of hemagglutinating (HA) and bactericidal (BCA) antibody activity in paired sera of recruits taken before and 1 month after vaccination with capsular polysaccharide of N. meningitidis group A. Sera marked with capital letters A to K are examined in detail in Table 4.*

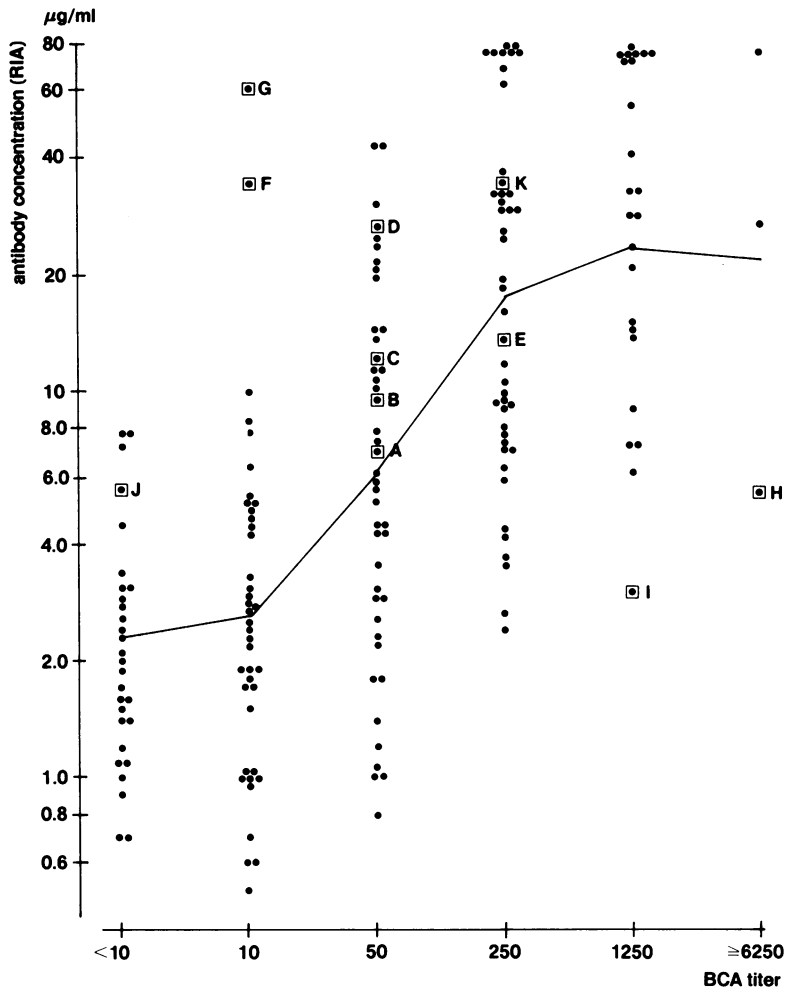

FIG. 6. *Correlation of bactericidal activity (BCA) and antibody concentration (by RIA) in paired sera of recruits taken before and 1 month after vaccination with capsular polysaccharide of N. meningitidis group A. Sera marked with capital letters A to K are examined in detail in Table 4.*

Most (72 of 76) sera that contained ≥8 µg of anti-MenA per ml had BCA titers exceeding 1:10, whereas four had low bactericidal activity. Two of these were preimmunization sera, and two were postimmunization sera. Two of these exceptional sera were further analyzed (F and G in Table 4). Their anti-MenA activity was present in all immunoglobulin classes including IgA. It is proposed that the IgA antibodies were responsible for the lack of bactericidal activity, in agreement with Griffis (18), who showed that IgA-class antibodies could block the bactericidal activity of other immunoglobulin classes. We have also seen a high proportion of IgA-class antibodies in sera of meningococcal meningitis patients taken at the acute stage of the infection, suggesting also a correlation with lack of protection (H. Käyhty, H. Jousimies-Somer, H. Peltola, and P. H. Mäkelä, submitted for publication).

ACKNOWLEDGMENTS

I thank Aino Siukonen and Raija Keijama for technical assistance, Kaija Helisjoki for secretarial work, and Seppo Sarna for advice in statistics.

This study was partly supported by Public Health Service contract no. 1AI-52502 from the National Institute of Allergy and Infectious Diseases, and by a grant from Merck Sharp & Dohme Research Laboratories.

LITERATURE CITED

1. **Anderson, P., R. B. Johnston, Jr., and D. H. Smith.** 1972. Human serum activities against *Hemophilus influenzae*, type b. J. Clin. Invest. 51:31-38.
2. **Anderson, P., R. B. Johnson, Jr., and D. H. Smith.** 1973. Methodology in detection of human serum anti-

bodies to *Hemophilus influenzae* type b, p. 87-94. *In* S. H. W. Sell and D. T. Karzon (ed.), *Hemophilus influenzae*. Vanderbilt University Press, Nashville, Tenn.

3. **Artenstein, M. S., B. L. Brandt, E. C. Tramont, W. C. Brache, Jr., H. O. Fleet, and R. L. Cohen.** 1971. Serological studies of meningococcal infection and polysaccharide vaccination. J. Infect. Dis. **124**:277-288.
4. **Artenstein, M. S., R. Gold, J. G. Zimmerly, F. A. Wyle, H. Schneider, and C. Harkins.** 1970. Prevention of meningococcal disease by group C polysaccharide vaccine. N. Engl. J. Med. **282**:417-420.
5. **Brandt, B. L., and M. S. Artenstein.** 1975. Duration of antibody responses after vaccination with group C *Neisseria meningitidis* polysaccharide. J. Infect. Dis. **131**: S69-S72.
6. **Brandt, B. L., M. S. Artenstein, and C. D. Smith.** 1973. Antibody responses to meningococcal polysaccharide vaccines. Infect. Immun. **8**:590-596.
7. **Brandt, B. L., F. A. Wyle, and M. S. Artenstein.** 1972. A radioactive antigen bindings assay for *Neisseria meningitidis* polysaccharide antibody. J. Immunol. **108**: 913-920.
8. **Erwa, H. H., M. A. Haseeb, A. A. Idris, L. Lapeyssonnie, W. R. Sanborn, and J. E. Sippel.** 1973. A serogroup A meningococcal polysaccharide vaccine. Studies in the Sudan to combat cerebrospinal meningitis caused by *Neisseria meningitidis* group A. Bull. W.H.O. **49**: 301-305.
9. **Frasch, C. E., and S. S. Chapman.** 1972. Classification of *Neisseria meningitidis* group B into distinct serotypes. I. Serological typing by microbactericidal method. Infect. Immun. **5**:98-102.
10. **Gold, R., and M. S. Artenstein.** 1971. Meningococcal infections. 2. Field trial of group C meningococcal polysaccharide vaccine in 1969-70. Bull. W.H.O. **45**: 279-282.
11. **Gold, R., J. L. Winklehake, R. S. Mars, and M. S. Artenstein.** 1971. Identification of an epidemic strain of group C *Neisseria meningitidis* by bactericidal serotyping. J. Infect. Dis. **124**:593-597.
12. **Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. J. Exp. Med. **129**:1307-1326.
13. **Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. II. Development of natural immunity. J. Exp. Med. **129**: 1327-1348.
14. **Gotschlich, E. C., I. Goldschneider, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. IV. Immunogenity of group A and group C meningococcal polysaccharides in human volunteers. J. Exp. Med. **129**:1367-1384.
15. **Gotschlich, E. C., T. Y. Liu, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. III. Preparation and immunochemical properties of the group A, group B and group C meningococcal polysaccharides. J. Exp. Med. **129**:1349-1365.
16. **Gotschlich, E. C., M. Rey, J. Etienne, W. R. Sanborn, R. Triau, and B. Cvjetanović.** 1972. The immunologic responses observed in field studies in Africa with group A meningococcal vaccines. Prog. Immunobiol. Stand. **5**: 485-491.
17. **Gotschlich, E. C., M. Rey, R. Triau, and K. J. Sparks.** 1972. Quantitative determination of human immune response to immunization with meningococcal vaccines. J. Clin. Invest. **51**:89-96.
18. **Griffis, J. M.** 1975. Bactericidal activity of meningococcal antisera: blocking by IgA of lytic antibody in human convalescent sera. J. Immunol. **114**:1779-1784.
19. **Kabat, E. A., and A. E. Bezer.** 1958. The effect on variation in molecular weight on the antigenicity of dextran in man. Arch. Biochem. **78**:306-318.
20. **Kasper, D. L., and F. A. Wyle.** 1972. Bactericidal antibody assay using ^{14}C-labeled *Neisseria meningitidis*. Proc. Soc. Exp. Biol. Med. **139**:1175-1180.
21. **Lepow, M. L., I. Goldschneider, R. Gold, M. Randolph, and E. Gotschlich.** 1977. Persistence of antibody following immunization of children with groups A and C meningococcal polysaccharide vaccines. Pediatrics **60**:673-680.
22. **Mäkelä, P. H., H. Käyhty, P. Weckström, A. Sivonen, and O.-V. Renkonen.** 1975. Effect of group A meningococcal vaccine in army recruits in Finland. Lancet **ii**: 883-886.
23. **Mäkelä, P. H., H. Peltola, H. Käyhty, H. Jousimies, O. Pettay, E. Ruoslahti, A. Sivonen, and O.-V. Renkonen.** 1977. Meningococcal group A and *Haemophilus influenzae* type b polysaccharide vaccines in children. A double blind field trial in Finland. J. Infect. Dis. **136**:S43-S50.
24. **Monto, A. S., B. L. Brandt, and M. S. Artenstein.** 1973. Response of children to *Neisseria meningitidis* polysaccharide vaccines. J. Infect. Dis. **127**:394-400.
25. **Peltola, H.** 1978. Group A meningococcal polysaccharide vaccine and course of the group A meningococcal epidemic in Finland. Scand. J. Infect. Dis. **10**:41-44.
26. **Peltola, H., P. H. Mäkelä, H. Käyhty, H. Jousimies, E. Herva, K. Hällström, A. Sivonen, O.-V. Renkonen, O. Pettay, V. Karanko, P. Ahvonen, and S. Sarna.** 1977. Clinical efficacy of meningococcus group A capsular polysaccharide vaccine in children three months to five years of age. N. Engl. J. Med. **297**:686-691.
27. **Roberts, R. B.** 1970. The relationship between group A and group C meningococcal polysaccharides and serum opsonins in man. J. Exp. Med. **131**:499-513.
28. **Taynay, A. E., P. A. Galvao, J. S. de Morais, E. C. Gotschlich, and R. A. Feldman.** 1974. Disease prevention by meningococcal serogroup C polysaccharide vaccine in preschool children: results after eleven months in Sao Paulo, Brazil. Pediatr. Res. **8**:429.
29. **Tramont, E. C., and M. S. Artenstein.** 1972. Latex agglutination test for measurement of antibodies to meningococcal polysaccharides. Infect. Immun. **5**:346-351.
30. **Wahdan, M. H., F. Rizk, A. M. El-Akkad, A. A. Ghoroyry, R. Hablas, N. I. Girgis, A. Amer, W. Boctar, J. E. Sippel, E. C. Gotschlich, R. Triau, W. R. Sanborn, and B. Cvjetanović.** 1973. A controlled field trial of a serogroup A meningococcal polysaccharide vaccine. Bull. W.H.O. **48**:667-673.
31. **Wahdan, M. H., S. A. Sallam, M. N. Hassan, A. A. Gawad, A. S. Rakha, J. E. Sippel, R. Hablas, W. R. Sanborn, N. M. Kassem, S. M. Riad, and B. Cvjecanović.** 1977. A second controlled field trial of a serogroup A meningococcal polysaccharide vaccine in Alexandria. Bull. W.H.O. **55**:645-651.
32. **Wong, K. H., O. Barrera, A. Sutton, J. May, D. H. Hochstein, J. D. Robbins, J. B. Robbins, P. D. Parkman, and E. B. Seligmann, Jr.** 1977. Standardization and control of meningococcal vaccines, group A and group C polysaccharides. J. Biol. Stand. **5**:197-215.
33. **World Health Organization Study Group.** 1976. Cerebrospinal meningitis control. Technical Report Series no. 588. World Health Organization, Geneva.