Comparison of Radioimmunoassay and Enzyme-Linked Immunosorbent Assay in Measurement of Antibodies to *Neisseria meningitidis* Group A Capsular Polysaccharide

E. COEN BEUVERY,^{1*} M. HELENA KAYHTY,² ALBERT B. LEUSSINK,¹ AND VISHNA KANHAI¹

Rijksinstituut voor Volksgezondheid en Milieuhygiene, 3720 BA Bilthoven, The Netherlands,¹ and National Public Health Institute, SF-00280 Helsinki 28, Finland²

Received 24 January 1984/Accepted 21 June 1984

Antibodies to meningococcal group A polysaccharide were determined by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) in serum samples from 16 adults vaccinated with bivalent meningococcal group A and C polysaccharide vaccine. The specific antibody levels in the serum samples were expressed as micrograms of antibody protein per milliliter of serum. For RIA the polysaccharide was radiolabeled extrinsically with ¹²⁵I. Both native polysaccharide and polysaccharide labeled with ¹²⁷I were used in ELISA. Because these antigens gave similar results, it can be concluded that the introduction of tyramine and iodine by the labeling procedure did not alter the antigenic activity of the polysaccharide. The reproducibility of RIA was clearly better than that of ELISA. The antibody levels detected by the methods were equal, which means that ELISA can be used satisfactorily to measure antibodies to meningococcal group A polysaccharide quantitatively. Some discrepant results were found due to an underestimation of immunoglobulin M antibodies in ELISA. This was shown by a correlation test in which a weakly significant negative correlation was found between the immunoglobulin M antibody level/immunoglobulin G antibody level ratio and the RIA antibody level/ELISA antibody level ratio.

Meningococcal diseases and immunization with meningococcal group A, C, W135, Y, and Z' (29E) polysaccharide vaccines result in the induction of antibodies (5, 6, 12-19, 21-24, 28, 33) in humans. These antibodies can be detected in serum samples obtained 1 week or more after the onset of the disease or injection of the vaccines. In adults the antibodies belong to the three main immunoglobulin isotypes, M (IgM), G, and A (5, 6, 14, 22, 23).

Many different serological methods have been used to measure the antibody response after both meningococcal infection and immunization: passive hemagglutination (1, 14, 21), latex agglutination (34), immunofluorescence (1), opsonization activity (31), bactericidal antibody assay (13), radioimmunoassay (RIA; 16, 21), and enzyme-linked immunosorbent assay (ELISA; 4, 6, 7, 27). Of these techniques only the RIA and the ELISA are appropriate to measure the amount of antibodies quantitatively.

Group A polysaccharide, which is used in RIA, was labeled extrinsically with ¹²⁵I. This label is introduced after treatment of the polysaccharide with cyanogen bromide and tyramine (2, 3, 16, 26). This method of labeling may alter the antigenic activity of the polysaccharide. However, this is contradicted by the good correlation obtained between antibodies measured by RIA and those measured by intact bacteria (21). We have studied this problem and present evidence that the introduction of the label to the polysaccharide does not change its antigenic activity. We also show results of a comparison of the two methods from measuring the total anti-group A polysaccharide antibody levels in 16 serum samples from adults immunized with a bivalent meningococcal group A and C polysaccharide vaccine. Although comparisons among several of the methods listed above have been published (1, 4, 7, 21, 30), RIA and ELISA have not been correlated. Moreover, the repeatability of both

techniques was studied by statistical evaluation of the results of both antibody assays done in duplicate on 3 consecutive days.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from 16 adult human volunteers who had been immunized with three lots of a bivalent meningococcal group A and C polysaccharide vaccine produced by the Rijksinstituut voor Volksgezondheid en Milieuhygiene. The characteristics of the three lots of vaccine were comparable and have been described elsewhere (6). Serum samples were collected 2 weeks after injection with vaccines and were stored at -70°C. The age of the vaccinated subjects ranged from 20 to 60 years.

Group A polysaccharide. Purified group A polysaccharide, used in the ELISA, was kindly provided by Rudy Tiesjema (Rijksinstituut voor Volksgezondheid en Milieuhygiene, Bilthoven, The Netherlands). The preparation met the World Health Organization minimal requirements (35, 36).

¹²⁵I and ¹²⁷I labeling of the tyramine derivative of group A polysaccharide. The tyramine derivative of group A polysaccharide was received from Emil Gotschlich (Rockefeller University, New York, N.Y.). The labeling of the derivative with both I isotopes was performed as described by Fraker and Speck (11).

Peroxidase-conjugated antibodies against the light chains of human immunoglobulins. IgG was isolated from normal human serum by DEAE-cellulose (in 0.01 M phosphate, pH 7.5) chromatography and was digested with pepsin (E. Merck AG, Darmstadt, West Germany) to obtain F(ab')₂ fragments (29). The fragments were purified by gel filtration on Sephadex G-100 in 0.01 M phosphate-buffered saline, pH 7.2. Antiserum against F(ab')₂ fragments, containing the light chains of immunoglobulins, was raised in sheep. The specificity of the antiserum was tested in a double-diffusion assay. IgG and F(ab')₂ fragments were used as antigen. The antiserum showed a complete identity with both antigens

* Corresponding author.

FIG. 1. Antigenic activity of group A polysaccharide (APS) and ^{125}I -labeled group A polysaccharide (APS-I) as measured in ELISA, using sheep antibodies to group A polysaccharide. OD, optical density.

The antiserum was processed and the antibody-peroxidase conjugate was prepared as described previously (6).

RIA. RIA was performed as described earlier (21).

ELISA procedures. (i) **Homologous ELISA for assessing the antigenic activity of ^{125}I -labeled group A polysaccharide.** The homologous ELISA was used for measuring the antigenic activity of the native and modified group A polysaccharides. The procedure and reagents have been described elsewhere (8).

(ii) **Assay of antibodies.** Both purified group A polysaccharide and ^{125}I -modified group A polysaccharide were used as antigens in the antibody assay (6, 7). In both assays purified group A polysaccharide was used for absorption of the serum samples (6). The peroxidase-conjugated antibodies were directed against the light chains of human immunoglobulins.

Calculation of antibody levels. Total antibody level (expressed as micrograms of antibody protein per milliliter) against group A polysaccharide was calculated by comparing the number of counts (RIA) or the corrected optical density (ELISA) of two dilutions of the serum samples with those of a dilution series of a human reference serum. This reference serum was a serum pool obtained from adults 2 weeks after immunization with group A polysaccharide vaccine. The pool contained 8.1 μg of IgM antibodies, 13.1 μg of IgG antibodies, and 2.7 μg of IgA antibodies per ml (total amount, 23.9 μg of antibody protein per ml). These antibody levels were determined by quantitative precipitation with group A polysaccharide and quantitative determination of IgM, IgG, and IgA present in the precipitate after the precipitate was redissolved (E. C. Beuvery, Ph.D. thesis, University of Amsterdam, Amsterdam, The Netherlands, 1980).

Statistical methods. Antibody levels were transformed to decimal logarithms and statistically evaluated by means of analysis of variance (32). Occasionally Spearman's rank correlation test (25) was applied.

RESULTS

Antigenic activities of group A polysaccharide and ^{125}I -labeled polysaccharide. Group A polysaccharide and ^{125}I -labeled group A polysaccharide were tested in the homologous ELISA, using sheep antibodies against the polysaccharides. The curves representing their antigenic activities are shown in Fig. 1. It can be concluded that the antigenic activities of both polysaccharide preparations were comparable.

Repeatability of RIA and both ELISAs. The antibody levels of 16 serum samples were assayed in duplicate by RIA and ELISA. Three consecutive assays were performed on each sample on separate days. In the ELISA both purified and ^{125}I -labeled group A polysaccharide were used as antigens. The coefficient of variation of the repeatability of the RIA was clearly lower than that of both ELISAs (13 versus 39%). Significant differences between the antibody levels were found on the three test days. This was largely due to a markedly high level shown on test day 3 by the ELISA in which ^{125}I -labeled group A polysaccharide was used.

Comparison of anti-group A polysaccharide ELISA and anti- ^{125}I -labeled group A polysaccharide ELISA. The results obtained by both ELISAs are shown in Fig. 2. For this purpose, the results from the serum samples in each system have been averaged by duplicate tests and test days. The data shown indicate that the ratio of antibody level found in

FIG. 2. Relationship between ELISA antibody levels to native (APS) and ^{125}I -labeled group A polysaccharide (APS-I) in 16 serum samples from adults after immunization with a bivalent group A and C polysaccharide vaccine. Symbols: (*) geometric mean of the antibody levels found in both ELISA systems; (---) diagonal through the geometric mean.

FIG. 3. Relationship between anti-group A polysaccharide RIA level and the combined anti-group A polysaccharide ELISA levels in 16 serum samples from adults after immunization with a bivalent group A and C polysaccharide vaccine. Symbols: (*) geometric mean of the antibody levels found in ELISA and RIA; (---) diagonal through the geometric mean.

the anti-group A polysaccharide ELISA to that found in the anti- ^{125}I -labeled group A polysaccharide ELISA does not depend upon the serum sample (individual). Indeed, the appropriate statistical test did not reveal a significant individual specificity. Because of these results we could combine the results of both ELISA systems. Moreover, the overall mean ratio of both anti-A polysaccharide levels could be meaningfully calculated. This overall mean ratio was 1.49, a value which is not significantly different from 1 (95% confidence limits, 0.60 and 3.70).

Comparison of RIA and ELISA results. Figure 3 represents comparison of the RIA and combined ELISAs. The RIA antibody level/averaged ELISA antibody level ratio depended upon the individual serum sample. About half of the individual samples had a ratio of roughly 1.6, whereas the other half had a ratio of about 0.6. This remarkable discrepancy was not related to the age of the vaccinated volunteers.

Comparison of IgM antibody/IgG antibody ratio and RIA antibody level/ELISA antibody level ratio. Immunization of adults with group A polysaccharide vaccine resulted in the induction of IgM, IgG, and IgA antibodies (5, 6, 14). The ratio of antibodies of these isotypes varies among individuals (5, 6). The discrepancy indicated in the preceding paragraph may be due to differences in sensitivity of both techniques for assaying the three isotype antibodies. This hypothesis was tested by comparing the IgM/IgG antibody level ratio (data taken from reference 8) and the RIA antibody level/ELISA antibody level ratio in the serum samples. Figure 4 indicates a weakly significant ($0.05 > P > 0.10$) negative rank correlation between both ratios.

DISCUSSION

Although the RIA has been the standard method for evaluation of the potential of the meningococcal polysaccharide vaccines for a long time in many laboratories (16, 21, 24), the more recently developed ELISA has been applied in its place (4, 6, 7, 27). Both techniques represent simple methods and seem to be very suitable because of their sensitivity (20). Both assays can also be used for measuring antibody levels in sera of small children, who may have low levels of specific antibody both before and after vaccination. These methods can be used to quantitatively calculate antipolysaccharide antibody levels. Results of both techniques appear to correlate rather well with those of the bactericidal antibody assay (7, 21).

Usually, by Farr-type RIA only total antibody levels are determined. The ELISA is a more appropriate way to separately measure antibody levels of the three main immunoglobulin isotypes. The disadvantages of the RIA are the hazards of using isotopes and the relatively expensive counting and monitoring devices. The disadvantage of the ELISA is the additional absorption step with purified polysaccharide required to eliminate the influence of nonspecific reactions. This step may be the cause of the lower repeatability of ELISA.

We found in the ELISA, using both "native" and labeled polysaccharide, that the introduction of iodine did not result in a change in the antigenic behavior of the polysaccharide (Fig. 1). The data presented in Fig. 2, however, suggest that the label may result in some loss of binding capacity of the treated polysaccharide. Because about 90% of the sugar residues of group A polysaccharide carry O-acetyl groups (9) and only vicinal hydroxylic groups are engaged in the reaction with cyanogen bromide, only about 10% of the sugar residues carry the iodine label. The nonterminal determinant of a linear polysaccharide is composed of about six sugar residues (10). The density of the iodine label may be too low to result in a change in antigenic activity of the polysaccharide.

In comparing RIA and ELISA two differences have been demonstrated. First, the repeatability of RIA was superior to that of ELISA. The coefficient of variation of the repeatability

FIG. 4. Relationship between the IgM antibody level/IgG antibody level ratio and the RIA antibody level/ELISA antibody level ratio in 16 serum samples from adults after immunization with a bivalent group A and C polysaccharide vaccine.

bility of our RIA was clearly lower than that described by Gold et al. (12). In addition, we found a weakly significant negative correlation between the IgM level/IgG level ratio and the RIA antibody level/ELISA antibody level ratio. Such a correlation may indicate differences in sensitivity of one or both techniques for assaying IgM and IgG antibodies. Gotschlich et al. (16) showed for six serum samples that the binding of labeled group A polysaccharide correlated very well with the logarithm of the antibody concentration. This suggests that the association constants of the antibodies in the six samples were identical. Most likely the relative contribution of antibodies of the three isotypes in the sample varied. As a consequence, the association constants of IgM, IgG, and IgA antibodies may not differ very much. We performed competition experiments with serum samples that greatly differed in the relative amounts of the three isotypes to group A polysaccharide. We also did these experiments with hyperimmune sheep antibodies. Our results suggested that the association constants of the antibodies in these serum samples and those of the three isotypes were comparable (E. C. Beuvery, R. W. van Delft, and J. Nagel, manuscript in preparation). Consequently, the discrepancy found in this study may indicate that the RIA does not depend on the relative contribution of IgM, IgG, or IgA antibodies. In the ELISA IgM antibodies will be underestimated in comparison with IgG antibodies. The difference may be due to a lower association constant of the conjugated antibodies against light chains for IgM than for IgG antibodies. The discrepancy may be eliminated by using a mixture of conjugated isotype-specific antibodies (30).

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service contract IAI-52502 from the National Institute of Allergy and Infectious Diseases. E.C.B. thanks the Council of Europe for fellowship 80-118 for a stay at the National Public Health Institute in Helsinki.

We thank José Dooper for preparing the manuscript.

LITERATURE CITED

1. 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.* 124:417-420.
2. Axen, R., and S. Ernback. 1971. Chemical fixation of enzymes to cyanogen halide activated polysaccharide carriers. *Eur. J. Biochem.* 18:351-360.
3. Axen, R., J. Porath, and S. Ernback. 1976. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature (London)* 214:1303-1305.
4. Basta, M. T., H. Russel, N. I. Guirguis, K. Hafez, and A. El Kholy. 1982. Enzyme-linked immunosorbent assay for determination of human antibodies to group C meningococcal polysaccharide (41299). *Proc. Soc. Exp. Biol. Med.* 169:7-11.
5. Beuvery, E. C. 1981. Immunization against bacterial meningitis. *J. Infect.* 3:S71-S79.
6. Beuvery, E. C., A. B. Leussink, R. W. van Delft, R. H. Tiesjema, and J. Nagel. 1982. Immunoglobulin M and G antibody responses and persistence of these antibodies in adults after vaccination with a combined meningococcal group A and group C polysaccharide vaccine. *Infect. Immun.* 37:579-585.
7. Beuvery, E. C., R. W. van Delft, and J. Nagel. 1983. ELISA of antibodies against capsular and non-capsular antigens of *Neisseria meningitidis*, p. 294-302. In G. P. Talwar (ed.), *Non-isotopic immunoassays and their applications*. Vikas Publishing House PVT LTD, New Delhi.
8. Beuvery, E. C., R. W. van Delft, R. H. Tiesjema, and J. Nagel. 1983. The enzyme-linked immunosorbent assay of meningococcal and some related *Escherichia coli* polysaccharides. *J. Biol. Stand.* 11:195-204.
9. Bundle, D. R., I. C. P. Smith, and H. J. Jennings. 1974. Determination of the structure and conformation of bacterial polysaccharides by carbon 13 nuclear magnetic resonance. Studies on the group-specific antigens of *Neisseria meningitidis* serogroups A and X. *J. Biol. Chem.* 249:2275-2281.
10. Cisar, J., E. A. Kabat, M. M. Dörner, and J. Liao. 1975. Binding properties of immunoglobulin combining sites specific for terminal or non-terminal antigenic determinants in dextran. *J. Exp. Med.* 142:435-459.
11. Fraker, P. J., and J. C. Speck. 1978. Protein and cell membrane iodinations with a sparingly soluble chloramide 1,3,4,6-Tetrachloro-3a,6a-diphenylglyccouiril. *Biochem. Biophys. Res. Commun.* 80:849-857.
12. Gold, R., M. L. Lepow, I. Goldschneider, T. F. Draper, and E. C. Gotschlich. 1978. Antibody responses of human infants to three doses of group A *Neisseria meningitidis* polysaccharide vaccine administered at two, four, and six months of age. *J. Infect. Dis.* 138:731-735.
13. Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein. 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. *J. Exp. Med.* 129:1327-1348.
14. Gotschlich, E. C., I. Goldschneider, and M. S. Artenstein. 1969. Human immunity to the meningococcus. IV. Immunogenicity 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 polysaccharide. *J. Exp. Med.* 129:1349-1365.
16. Gotschlich, E. C., M. Rey, R. Triau, and K. J. Sparks. 1972. Quantitative determination of the human immune response to immunization with meningococcal vaccines. *J. Clin. Invest.* 51:89-96.
17. Griffiss, J. M., B. L. Brandt, P. L. Altieri, G. B. Pier, and S. L. Berman. 1981. Safety and immunogenicity of group Y and group W135 meningococcal capsular polysaccharide vaccines in adults. *Infect. Immun.* 34:725-732.
18. Griffiss, J. M., B. L. Brandt, P. L. Altieri, G. B. Pier, and S. L. Berman. 1983. Safety and immunogenicity of a group 29E meningococcal capsular polysaccharide vaccine in adults. *Infect. Immun.* 39:247-252.
19. Griffiss, J. M., B. L. Brandt, and D. D. Broude. 1982. Human immune response to various doses of group Y and group W135 meningococcal polysaccharide vaccines. *Infect. Immun.* 37:205-208.
20. Hill, H. R., and J. M. Matsen. 1983. Enzyme-linked immunosorbent assay and radioimmunoassay in the serologic diagnosis of infectious diseases. *J. Infect. Dis.* 147:258-263.
21. Kayhty, H. 1980. Comparison of passive hemagglutination, bactericidal activity, and radioimmunological methods in measuring antibody responses to *Neisseria meningitidis* group A capsular polysaccharide vaccine. *J. Clin. Microbiol.* 12:256-263.
22. Kayhty, H. 1982. The unspecific antibody response to *N. meningitidis* group A capsular polysaccharide often seen in bacterial diseases. *Parasite Immunol.* 4:157-170.
23. Kayhty, H., H. Jousimies-Somer, H. Peltola, and P. H. Makela. 1981. Antibody response to capsular polysaccharides of groups A and C *Neisseria meningitidis* and *Haemophilus influenzae* type b during bacteremic disease. *J. Infect. Dis.* 143:32-41.
24. Kayhty, H., V. Karanko, H. Peltola, S. Sarna, and P. H. Makela. 1980. Serum antibodies to capsular polysaccharide vaccine of group A *Neisseria meningitidis* followed for three years in infants and children. *J. Infect. Dis.* 142:861-868.
25. Kendall, M. G. 1948. Rank correlation methods. Griffin and Co., London.
26. Kohn, J., and M. Wilchek. 1981. Procedures for the analysis of cyanogen bromide-activated sepharose of sephadex by quantitative determination of cyanate esters and imidocarbonates. *Anal. Biochem.* 105:375-382.
27. Leinonen, M., and C. E. Frasch. 1982. Class-specific antibody response to group B *Neisseria meningitidis* capsular polysaccharide: use of polylysine precoating in an enzyme-linked

- immunosorbent assay. *Infect. Immun.* **38**:1203–1207.
28. **Makela, P. H., H. Peltola, H. Kayhty, H. Jousimies, O. Pettay, E. Ruoslahti, A. Sivenon, and O.-V. Renkonen.** 1977. Polysaccharide vaccines of group A *Neisseria meningitidis* and *Haemophilus influenzae* type b: a field trial in Finland. *J. Infect. Dis.* **136**:S43–S50.
29. **Nisonoff, A., F. C. Wissler, and L. N. Lipman.** 1960. Properties of the major component of a peptic digest of rabbit antibody. *Science* **132**:1770–1771.
30. **Pedersen, F. K., J. Henrichsen, and G. Schiffman.** 1983. Comparison of enzyme-linked immunosorbent assay and radioimmunoassay for determination of anti-pneumococcal polysaccharide antibodies. *Acta Pathol. Microbiol. Immunol. Scand. Sect. C* **91**:251–255.
31. **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.
32. **Scheffé, H.** 1959. The analysis of variance. John Wiley & Sons, Inc., New York.
33. **Tiesjema, R. H., and E. C. Beuvery.** 1978. Immunogeniteit van serogroep B, 29E, W135 en Y meningococcal polysaccharide-vaccins. *Min. Volksgezond. Milieuhyg. Versl. Adv. Rapp.* **40**:250–252.
34. **Tramont, E. C., and M. S. Artenstein.** 1972. Latex agglutination test for measurement of antibodies to meningococcal polysaccharides. *Infect. Immun.* **5**:346–351.
35. **World Health Organization Expert Committee on Biological Standardization.** 1976. Requirements for meningococcal polysaccharide vaccine. W.H.O. Tech. Rep. Ser. **549**:50–75.
36. **World Health Organization Expert Committee on Biological Standardization.** 1981. Requirements for meningococcal polysaccharide vaccine. W.H.O. Tech. Rep. Ser. **658**:174–184.