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# Human Antibody Response to a Group B Serotype 2a Meningococcal Vaccine Determined by Immunoblotting

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**The antibody response of 30 volunteers vaccinated with a complex of group B polysaccharide and outer membrane vesicles (OMV) from serotype 2a *Neisseria meningitidis* and of 3 individuals who received a placebo vaccine was determined by immunoblotting. OMV were separated by sodium dodecyl sulfate-gel electrophoresis and electrotransferred to nitrocellulose filters. Binding of immunoglobulin G (IgG), IgA, and IgM antibodies in the human sera to OMV components was detected with class-specific peroxidase-conjugated antibodies. The immunoblotting results were also related to the bactericidal activity of the sera and the meningococcal carrier status of the volunteers. Before vaccination weakly reactive bands in the molecular weight range of 140,000 to 10,000 were observed on the blots. Sera from carriers showed more marked bands. Individual patterns of increased reactivity were seen 6 weeks after vaccination. The main immunoreactive components of OMV corresponded to a molecular weight of 43,000 (class 1 protein), 30,000 (class 5 proteins), and 22,000. IgG antibodies in postvaccination sera of high bactericidal titers showed distinct binding to the 43,000-molecular-weight antigen. Meningococcal carriers had antibodies against an antigen of 22,000 molecular weight; in polyacrylamide gels this component did not stain with Coomassie brilliant blue or silver. The marked binding of IgG antibodies to the class 5 proteins decreased considerably between weeks 6 and 25 after vaccination. Periodate oxidation of OMV abolished the binding of IgG antibodies to the class 5 proteins, whereas the antigenicity of the 43,000-molecular-weight (class 1 protein) and 22,000-molecular-weight antigens was unaffected.**

A meningococcal vaccine trial was carried out in Norway in 1981 and 1982. Thirty-three adults were given a vaccine consisting of noncovalent complexes of group B polysaccharide and outer membrane material from serotype 2a meningococci, whereas 27 volunteers received the placebo vaccine (6). The immune response of the individuals was evaluated by determination of the antibody levels (18) and the bactericidal activity of the sera (6; L. O. Frøholm, unpublished data). In the present communication, the antibody response was studied by an immunoblotting method. With this technique we wanted to investigate the immunogenicity of the different components in the outer membrane fraction in the vaccine and, if possible, to correlate the results with previous determinations of the immune response such as antibody levels and bactericidal activities of the sera (6, 18; Frøholm, unpublished data). It was also of interest to see if the meningococcal carrier status of the volunteers would influence the pattern of reactive bands on the immunoblots.

(The preliminary findings with sera from 15 of the vaccinees have been reported previously (E. Wedege, L. O. Frøholm, T. E. Michaelsen, and C. E. Frasch, The Pathogenic Neisseriae. Proceedings of the Fourth International Symposium, 1984, in press.)

## MATERIALS AND METHODS

**Vaccination sera.** The volunteers received a vaccine dose of 0.5 ml consisting of: outer membrane vesicles (OMV) from *Neisseria meningitidis* M986 NCV-1 serotype 2a, 50 μg of protein and 8 μg of lipopolysaccharide (LPS); *N. meningitidis* group B polysaccharide, 50 μg; sodium thimerosal, 50 μg; lactose, 3 mg; and NaCl, 4.25 mg (lot 790626VB, obtained from C. E. Frasch, Center for Drugs and Biologics, Bethesda, Md.) (6). An identical dose was given 4 weeks after the first immunization. The placebo controls received sodium thimerosal, lactose, and NaCl only. The sera used for immunoblotting were those collected from the individuals at the day of the first vaccination (prevaccination sera) and after 6 and 25 weeks. They were stored at −25°C. Sera from 33 volunteers, 30 of the 33 vaccinees and 3 of the 27 placebo controls, were selected for the present investigation. Study of pharyngeal swabs of bacterial isolates showed that 55% of these individuals were meningococcal carriers at the time of vaccination (6). The bactericidal titers used in this report were determined with human complement (Frøholm, unpublished data). The numbers designating the volunteers are the same as published previously (6).

**SDS-gel electrophoresis.** The antigen in the immunoblotting experiments was an OMV fraction isolated from the same meningococcal strain as the vaccine OMV antigen (5). Its protein content was measured by the Folin method (11) with bovine serum albumin as the standard. OMV were boiled for 5 min in sample buffer with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol and separated in a discontinuous SDS-gel electrophoretic system (10). The samples contained pyronine B as a marker for the subsequent transblot electrophoresis. The separating and stacking gels contained 12 and 4% acrylamide, respectively. The dimensions of the 1.5-mm-thick gels were 14 by 12 cm. Electrophoresis through the separating gel was performed at 30 mA for 3 h in cold buffer. The proteins used as molecular weight standards were: phosphorylase *a* (94,000), bovine serum albumin (67,000), ovalbumin (45,000), lactate dehydrogenase (36,000), car-

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bonic anhydrase (30,000), chymotrypsinogen A (25,000), RNase (14,000), and cytochrome *c* (12,000). SDS-gels were stained with Coomassie brilliant blue (13).

**Detection of antibodies by immunoblotting.** After SDS-gel electrophoresis, the separated OMV were transferred to nitrocellulose (NC) filters (0.45-μm pore size; Bio-Rad Laboratories, Richmond, Calif.) at 300 to 400 mA for 3 h, using cold Tris glycine buffer with 20% methanol (22). The blots were then cut into 0.5-cm-wide strips, each corresponding to approximately 5 μg of OMV protein before gel electrophoresis. Guide strips were stained with amido black (7). The other strips were soaked in a blocking buffer of 3% bovine serum albumin in physiological saline for 30 min before incubation overnight in pre- or postvaccination sera diluted 1:200 with the blocking buffer. Afterwards the blots were treated for 2 h with peroxidase-conjugated rabbit anti-human immunoglobulin G (IgG) (γ chains), IgA (α chains), or IgM (μ chains) (DAKOPATTS a/s, Denmark), diluted 1:500 with the blocking buffer. The strips were washed four times in physiological saline after each step. Binding of the enzyme-labeled antibodies was detected with 3-amino-9-ethylcarbazole and hydrogen peroxide (14). The blots with anti-IgG, anti-IgA, and anti-IgM antibodies were stained for 4, 10, and 20 min, respectively, and then rinsed in water and dried. The incubations and washings were performed at room temperature on a rotary shaker. No binding of the peroxidase-conjugated antisera to OMV was observed in control experiments.

A mouse monoclonal antibody, 3-1-P1.2 (provided by W. D. Zollinger) that reacted with class 1 protein from serotype 2 *N. meningitidis* strains (27) was used to identify this protein on NC strips. OMV were separated and electroblotted as described above and incubated overnight with the monoclonal antibody (1:1,000), followed by a 2-h incubation with peroxidase-conjugated sheep anti-mouse immunoglobulin (1:10,000) (provided by T. E. Michaelsen). The monoclonal antibody was omitted in the control during the overnight incubation. The antibodies in these studies were diluted with the same blocking buffer as described above.

**Periodate oxidation of OMV.** Periodate oxidation was carried out in the dark for 5 h at 33°C in 0.05 M sodium acetate (pH 5.0) as described by Spiro (19). The final concentration of sodium periodate was 0.07 or 0.035 M. The oxidation was stopped by adding an excess of ethylene glycol. The samples were dialyzed overnight in physiological saline before SDS-gel electrophoresis. In one control, OMV were added after the reduction of the periodate by ethylene glycol; in another, OMV were incubated for 5 h at 33°C in sodium acetate buffer without periodate to examine the stability of the antigen.

**Statistical analysis.** Student's *t* test was used to evaluate the significance of the differences in the serum antibody levels which had previously been measured by the enzyme-linked immunosorbent assay (ELISA) (18). Differences were considered to be significant when *P* values less than 0.05 were found.

## RESULTS

**Analysis of OMV.** SDS-gels of the OMV preparation used in the vaccine and as immunoblotting antigen showed two main and two minor stained bands (Fig. 1, lane b). These bands corresponded from top to bottom to the class 1, 2, 4, and 5 proteins of the major outer membrane proteins (25) and were found to have molecular weights of 43,000, 41,000, 34,000, and 30,000, respectively. A similar pattern of bands

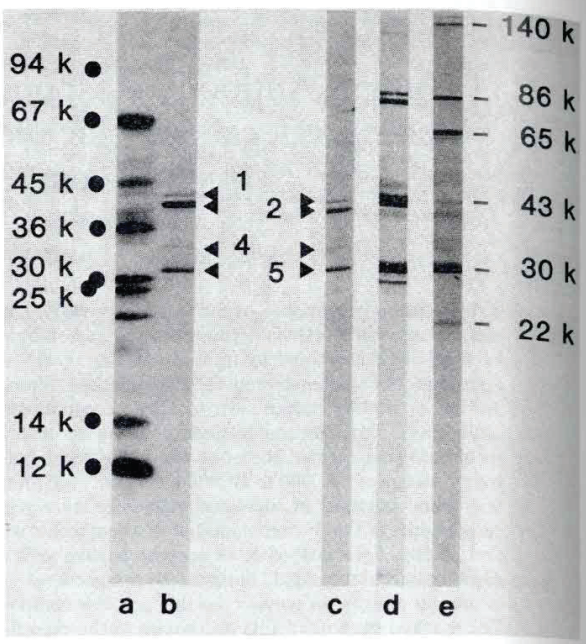



FIG. 1. SDS-gel electrophoretic and immunoblot analyses of OMV from *N. meningitidis*. SDS-gel of standard proteins (lane a) and OMV (lane b) stained with Coomassie brilliant blue. The dots mark the positions of the standard proteins used (from top to bottom: phosporylase *a*, bovine serum albumin, ovalbumin, lactate dehydrogenase, carbonic anhydrase, chymotrypsinogen A, RNase, and cytochrome *c*). Lane c: NC strip with electroblotted OMV stained with amido black. The protein classes 1, 2, 4, and 5 of OMV are marked with arrows. Lanes d and e: Immunoblots showing the different patterns of IgG antibody binding to the OMV with two individual sera (1:200) drawn 6 weeks after vaccination. The molecular weights of the main antigenic components are indicated to the right (k, $10^3$). The immunoreactive bands were visualized with peroxidase-conjugated anti-human IgG (1:500).

was seen after electrotransfer to NC filters (Fig. 1, lane c). Blots incubated with 6-week postvaccination sera showed a more complex pattern of antigenic OMV components, and the individual sera seemed to recognize different antigens (Fig. 1, lanes d and e). IgG antibodies bound most markedly to OMV components that corresponded to molecular weights of 140,000, 86,000, 65,000, 43,000 (class 1 protein), 30,000 (class 5 proteins), and 22,000. The antigens of 140,000, 86,000, and 65,000 molecular weight, but not the 22,000-molecular weight antigen, were also observed as very faintly stained bands on SDS-gels.

**IgG antibody response after vaccination.** Figure 2 demonstrates the binding of IgG antibodies in sera collected 6 weeks after vaccination of the 33 volunteers in this study. The patterns of reactive bands varied considerably from individual to individual. The majority of the sera showed the strongest binding to the 30,000-molecular-weight antigen, but other OMV components of higher and lower molecular weight as described above were also recognized. Weakly stained bands were observed with sera from volunteers 1, 31, and 45, who only received one meningococcal vaccine dose, and with sera from the placebo controls 34 and 82.

IgG antibodies in the sera from volunteers 2, 42, 71, 87, 111, and 112 bound distinctly to the 43,000-molecular-weight antigen (Fig. 2). The bactericidal titers of these sera were

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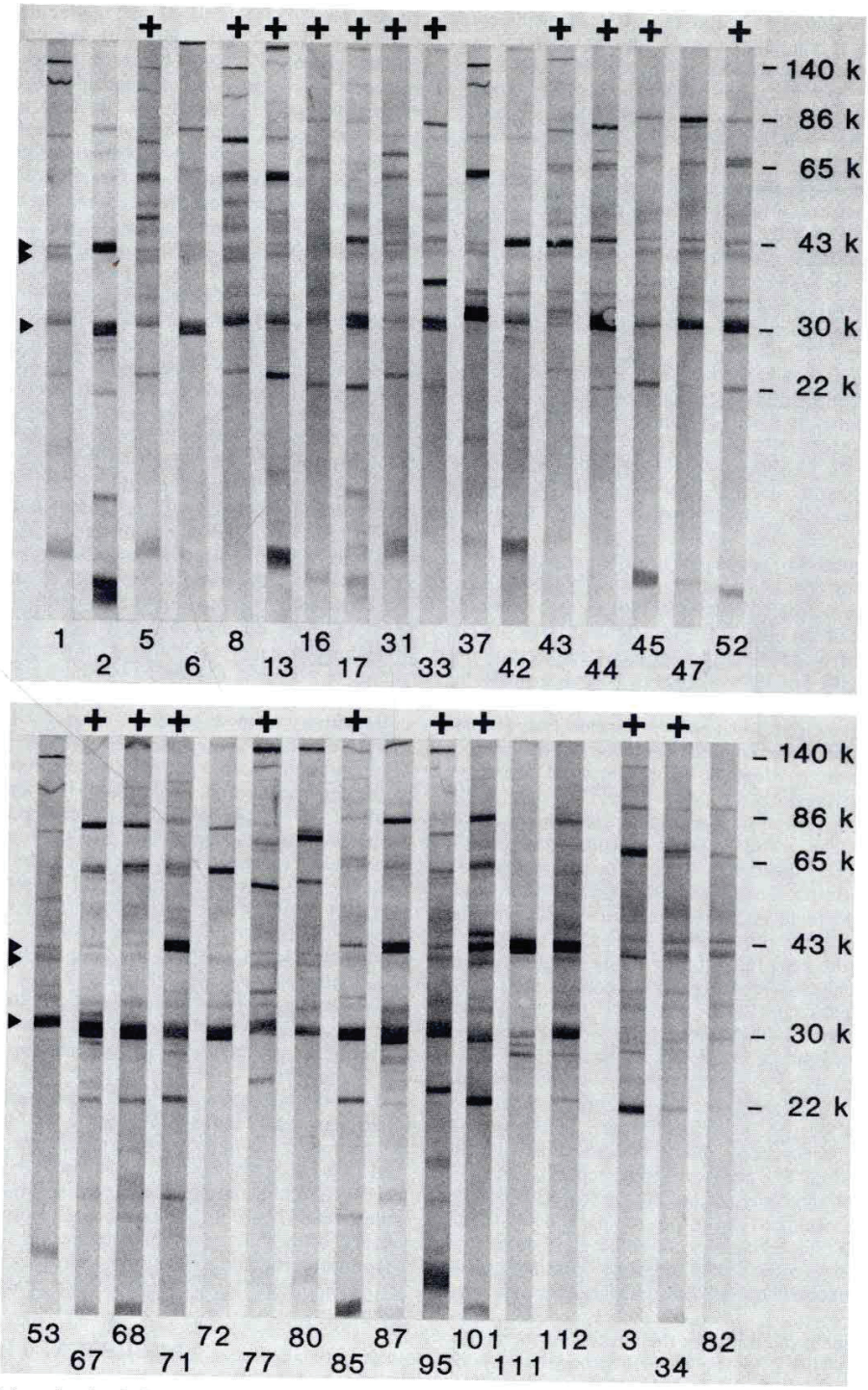



FIG. 2. Immunoblots showing IgG antibody activity against OMV from *N. meningitidis* in postvaccination sera from 30 vaccinees and 3 placebo controls. OMV were separated by SDS-gel electrophoresis, electroblotted to NC filters, and incubated with sera (1:200) drawn 6 weeks after the first vaccination. IgG antibody binding was visualized with peroxidase-conjugated anti-human IgG (1:500). The volunteer number designation is marked at the bottom of each strip; the + on the top of the strip indicates that the volunteer was a meningococcal carrier during the first 6 weeks after vaccination. The arrows to the left show (from top to bottom) the protein classes 1, 2, and 5, respectively. The molecular weights of the main antigenic components are seen to the right (k, $10^3$). The strips represent several experiments, leading to some variations in the mobilities of the main bands. Volunteers 3, 34, and 82 were placebo controls, whereas 1, 31, and 45 only received one dose of meningococcal vaccine.

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1:64, 1:128, 1:64, 1:256, 1:512, and 1:>1,024, respectively. Only two sera of similar high activity did not detect this antigen (numbers 52 and 67 with titers of 1:512 and 1:64, respectively). The IgG antibodies from vaccinees 17, 43, and 101 showed moderate binding to the 43,000-molecular-weight antigen. The bactericidal titers of these sera ranged from 1:8 to 1:32.

Many of the 6-week postvaccination sera had IgG antibodies directed against an antigen of 22,000 molecular weight (Fig. 2). The antigen was not stained in SDS-gels with Coomassie brilliant blue or silver (24) or on NC filters with amido black. The presence of this band on immunoblots seemed to be correlated with meningococcal carriage of the volunteers as it was generally weaker or not detected with sera from noncarriers.

In Fig. 2 it can also be seen that several sera recognized low-molecular-weight components in the range about 10,000 to 8,000. These antigens probably represented lipopolysaccharides (LPS) in the OMV fraction (23). This assumption was supported by the detection of bands of similar molecular weight in SDS-gels after staining with alcian Blue or Stains-All (Eastman Kodak Co., Rochester, N.Y.) (E. Wedege, unpublished data).

**IgA and IgM antibody responses after vaccination.** The binding to OMV of IgA and IgM antibodies in the 6-week postvaccination sera from all volunteers was also studied. The results for six of the individuals are shown in Fig. 3, and they corresponded to previous findings (Wedege et al., in press). IgA antibodies in 40% of all sera bound strongly to one or more of the 140,000-, 86,000-, 43,000-, 30,000-, and 22,000-molecular-weight antigens, whereas the others showed weak bands. Marked IgA-reactive bands on the blots did not seem to depend on bactericidal titers or meningococcal carriage.

IgM antibodies in 30% of the sera recognized one or more of the 43,000-, 30,000-, and 22,000-molecular-weight antigens. The majority of these sera were from meningococcal carriers. No relationship with the bactericidal titers was observed. The IgM-reactive bands were usually less strong than those seen with the other antibody classes. Compared with the IgA antibodies, the IgM antibodies bound more often to LPS antigens but less often to antigens of molecular weights above 43,000.

**Periodate oxidation of OMV.** When OMV were oxidized with periodate, the binding of IgG antibodies to the 43,000- and 22,000-molecular-weight antigens was unaffected, whereas that to the 30,000-molecular-weight antigen was reduced or abolished (Fig. 4, lanes c to f). Often several weak bands were observed in the 30,000-molecular-weight region after oxidation. The periodate treatment also affected the antigenicity of the components of 86,000, 65,000, and 34,000 molecular weight (class 4 protein) and the LPS antigens. The two concentrations of sodium periodate used were equally effective, and the antibody binding to the controls was similar to that of the untreated sample (see above).

Periodate treatment did not alter the binding of the monoclonal antibody 3-1-P1.2 to the class 1 protein of 43,000 molecular weight (Fig. 4, lanes g and h). However, both this band and that obtained after incubation with a serum of high bactericidal activity were somewhat more diffuse after oxidation (compare lanes e and f and lanes g and h in Fig. 4).

SDS-gels stained with Coomassie brilliant blue showed only faint traces of the 30,000-molecular-weight protein after periodate treatment of OMV (Fig. 4, lanes a and b). The 34,000-molecular-weight protein was fainter, whereas the proteins of 43,000 and 41,000 molecular weight stained equally well after oxidation. The staining intensity of the 30,000-molecular-weight band in the two controls (see above) was similar to that of untreated OMV, indicating that no visible proteolysis of this protein took place during incubation and dialysis.

**Comparison of antibody distribution in pre- and postvaccination sera.** The effect of vaccination was studied by comparing the binding patterns of each antibody class before and 6 weeks after vaccination of the 33 volunteers. The results for volunteers 13, 85, and 87 have been reported previously (Wedege, et al., in press). The blots for six other volunteers, two of whom received the placebo vaccine (numbers 3 and 34), are presented in Fig. 3. The figure also shows the IgG antibody activity of sera drawn 25 weeks after the first vaccination. Generally, the prevaccination sera showed faint bands on the blots that corresponded to the major outer membrane proteins of molecular weights of 43,000, 41,000, 34,000, and 30,000, but other weak bands in the molecular weight range of 140,000 to 10,000 were also observed. Several prevaccination sera demonstrated more distinct binding of IgG, IgA, or IgM antibodies (see numbers 68, 71, and 80 in Fig. 3 as examples); 69, 67, and 82% of such sera, respectively, were from meningococcal carriers. No relationship to the bactericidal titers of the sera was noted. Increased antibody binding to many of the antigens was seen 6 weeks after vaccination (Fig. 3). As described above, the 30,000-molecular-weight antigen seemed to be most antigenic, in addition to the 43,000-molecular-weight antigen recognized by IgG antibodies in the serum of high bactericidal activity from volunteer 71.

Figure 3 also shows that the prevaccination sera from volunteers 34, 68, and 71, who were meningococcal carriers at the time of the first vaccination, had antibodies of one or more of the immunoglobulin carrier classes to the 22,000-molecular-weight antigen. The possible relationship between carriage and this band is further demonstrated by referring to individuals 3 and 80 in the figure. Volunteer 3 became a carrier 4 weeks after the first vaccination, and volunteer 80 became a carrier sometime between weeks 6 and 25. The blots showed IgG antibodies in their corresponding postvaccination sera that bound distinctly to the 22,000-molecular-weight antigen. After the placebo vaccination of volunteers 3 and 34, the staining intensities of the other bands did not increase, in contrast to that observed for the vaccinees 53, 68, 71, and 80.

**Time-related decrease in antibody binding to the 30,000-molecular-weight antigen.** When the binding of IgG antibodies to the 30,000-molecular-weight antigen was compared 6 and 25 weeks after vaccination, a marked decrease with time was seen in the staining intensity of this band with sera from volunteers 53, 68, 71, and 80 (Fig. 3). Similar findings were observed with all sera that showed strong IgG antibody response to the antigen 6 weeks after vaccination. The other antigens did not demonstrate a corresponding decrease with time.

## DISCUSSION

Immunoblotting has found wide applications in the study of bacterial and viral infections (as reviewed by Towbin and Gordon [21]). We used the method to investigate the immune response in human volunteers who received a group B serotype 2a meningococcal vaccine (6). OMV in the vaccine were resolved by SDS-gel electrophoresis and electrotransferred to NC filters for immunoperoxidase staining with sera from 33 volunteers. The use of secondary

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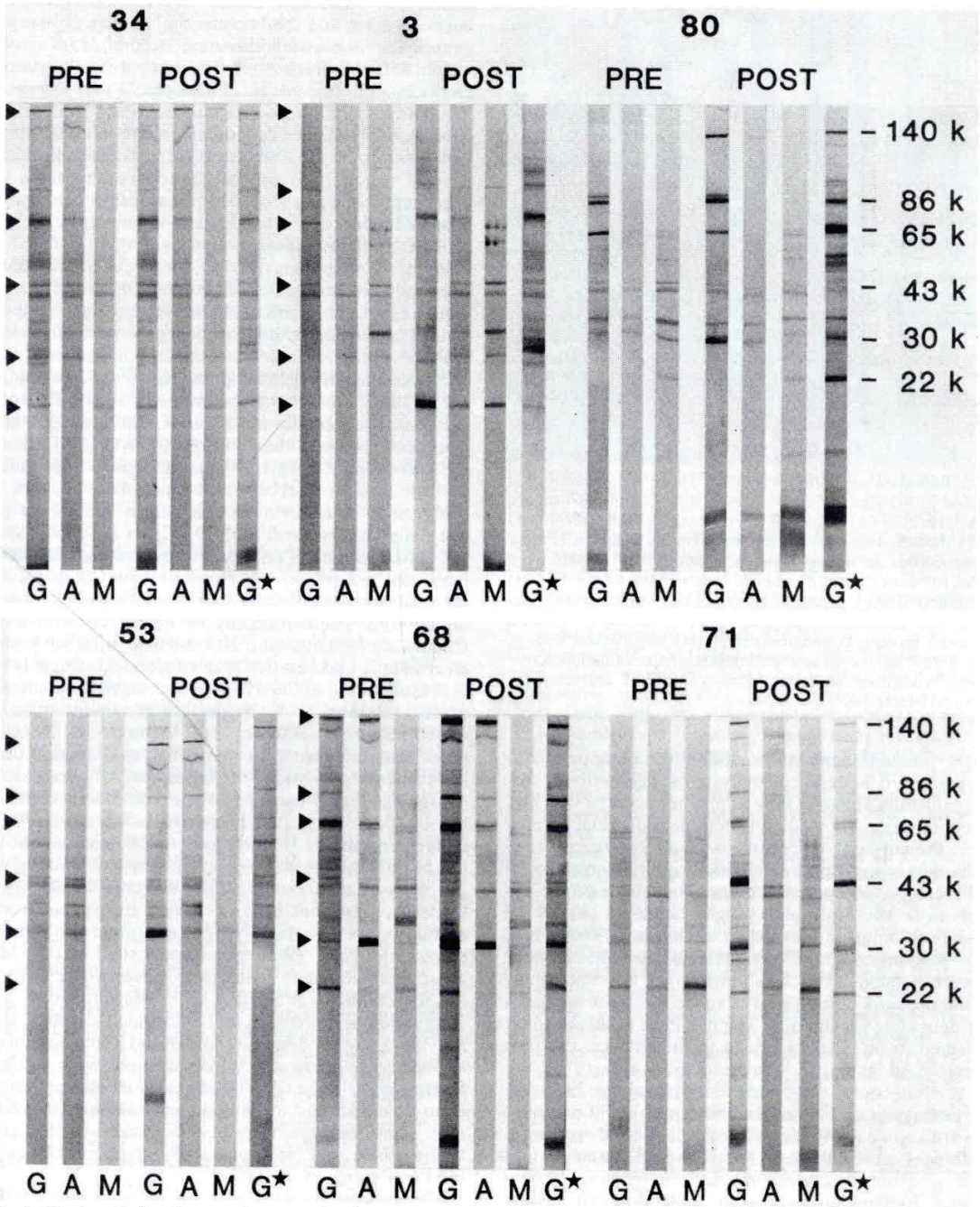



FIG. 3. Antibody activity of pre- and postvaccination sera against OMV components from *N. meningitidis*. The binding to OMV of IgG, IgA, and IgM antibodies in pre (PRE)- and post (POST)-vaccination sera (1:200) is marked by G, A, and M, respectively, at the bottom of the strips. The postvaccination sera were collected 6 weeks after the first vaccination. G* represents IgG antibody binding of the 25-week postvaccination sera. The volunteer number designation is shown on top of the blots. The molecular weights of the main antigenic components of OMV are indicated to the right (k, $10^3$), and the arrows show the corresponding bands for each volunteer. The immunoblots were from several experiments, causing some variation in the mobilities of the main antigens. Strips showing IgG antibody binding of the 6- and 25-week postvaccination sera from each volunteer were from the same experiment. Volunteers 3 and 34 received the placebo vaccine, whereas 53, 68, 71, and 80 were vaccinated twice. Volunteer 53 did not carry meningococci (see Results).

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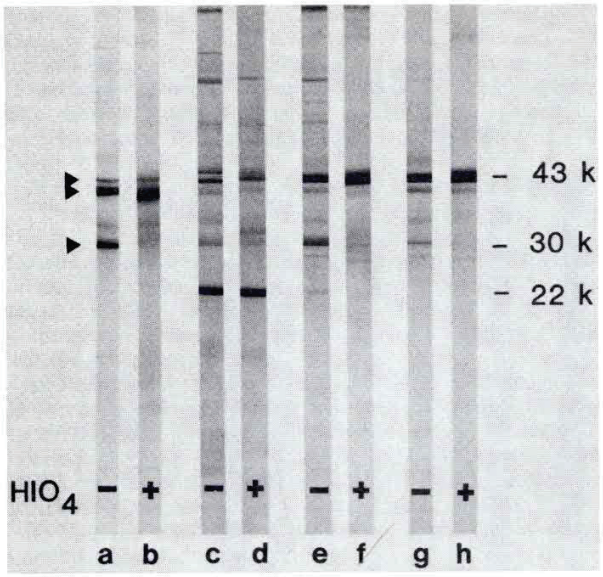



FIG. 4. Periodate oxidation of OMV from *N. meningitidis*. Lanes a and b: SDS-gels of OMV stained with Coomassie brilliant blue. The class 1, 2, and 5 proteins are indicated with arrows (top to bottom) to the left. Lanes c to f: Immunoblots showing the binding of IgG antibodies in 6-week postvaccination sera (1:200) from volunteers 101 (lanes c and d) and 112 (bactericidal titer 1:>1,024) (lanes e and f). Lanes g and h: Immunoblots demonstrating the binding of the monoclonal antibody 3-1-P1.2 (1:1,000) directed against class 1 protein. Periodate-treated OMV are marked with +. All strips were from the same experiment, and the immunoreactive bands were visualized with peroxidase-conjugated antisera (see Materials and Methods).

antibodies directed against the different immunoglobulin classes permitted a detailed analysis of the antibody response against the various OMV components in the vaccine.

The immunoblots showed considerable individual heterogeneity in the antibody response 6 weeks after vaccination. IgG antibodies in some postvaccination sera recognized only one OMV component, whereas other sera detected up to six components in the molecular weight range of 140,000 to 10,000. Fewer antigens seemed to elicit the IgA and IgM antibody response. The 30,000-molecular-weight antigen, which corresponded to the class 5 proteins of the major outer membrane proteins (25), appeared to be the most immunogenic protein after vaccination. The presence of this band on blots seemed to be independent of meningococcal carriage and bactericidal activity. Weak antibody activity to the protein was observed in the individuals receiving only one vaccination dose and in the placebo controls. The class 5 proteins are known to induce a strong IgG antibody response after meningococcal infection (16). However, Zollinger and Mandrell (26) obtained evidence for the response being lower after vaccination than after infection. Our results suggested that the antibody levels against these proteins decreased with time after vaccination. This and the fact that the class 5 proteins are variable (15) probably indicate that they are of less importance as immunogens in a vaccine.

One of our main findings was the strong IgG antibody binding to the 43,000-molecular-weight antigen with sera of high bactericidal activity. This was observed with six of the eight sera of titers >1:64, and five of these also had IgA antibodies directed against the protein. The antigen corresponded to the class 1 protein, which was confirmed with the monoclonal antibody 3-1-P1.2 (Fig. 4). It is therefore tempting to suggest that the bactericidal activity of many sera is caused by antibodies directed to the 43,000-molecular-weight antigen. This assumption is supported by studies with monoclonal antibodies (L. F. Mocca, A. B. Karpas, L. H. Wu, and C. E. Frasch, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 295, 1984) and the observation of antibody binding to the class 1-class 2 protein region on blots with one human serum of high bactericidal activity (26). The relationship between class 1 protein antibodies and bactericidal activity may be important in the formulation of new vaccines.

Periodate treatment of OMV did not affect the binding of IgG antibodies to the class 1 protein. Therefore, the majority of the epitopes on this antigen reacting with antibodies in sera of high bactericidal activity were probably not carbohydrates or amino acids susceptible to oxidation (2, 19). The same conclusion might be drawn for the single epitope that reacted with the monoclonal antibody 3-1-P1.2. In contrast, the class 5 protein did not bind IgG antibodies or stain with Coomassie brilliant blue after oxidation (Fig. 4). Cleavage of the protein might explain these findings, but peptide bonds are not likely to be broken by periodate (2, 19). LPS is reported to associate with some outer membrane proteins even after boiling with SDS (9, 17). If LPS interacted with the class 5 proteins, and the corresponding band of 30,000-molecular weight on the blots was due to IgG antibodies directed primarily against LPS, then the antigenicity of the bound LPS would probably be destroyed after oxidation. Coomassie brilliant blue is known to bind to proteins by electrostatic and van der Waals forces (3), and it is possible that the binding of the dye was prevented by changes in the protein molecule after periodate treatment. The control experiments (see Materials and Methods and Results) indicated that the class 5 protein was not degraded under the experimental conditions in the absence of periodate.

Meningococcal carriage of the volunteers seemed to be associated with antibody activity to an antigen of 22,000 molecular weight. The reactivity of the component was not destroyed by periodate. The antigen had unusual staining properties because it was not detected in SDS-gels with Coomassie brilliant blue or silver. Its gel electrophoretic mobility was not modified by treatment with heat or 2-mercaptoethanol (Wedege, unpublished data). Many of these characteristics resembled those of the CA2 antigen (W. D. Zollinger, J. S. Ray, E. E. Moran, and R. Seid, 4th International Conference on Pathogenic Neisseria, abstr. no. 58, 1984), the H.8-binding antigen (1), and an antigen described by Sugasawara (20) in meningococcal strains. Antibodies against pili of 16,000 to 21,000 molecular weight have been demonstrated in patients with meningococcal disease (16). These antigens were also not stained with Coomassie brilliant blue, but, in contrast to our findings, they did not bind antibodies of the IgG class (16).

Immunoblotting is not a strictly quantitative method, but it gives some indications of the relative antibody levels present and the antibody avidity (8). Each NC strip in our experiments corresponded to 5 μg of OMV protein applied before gel electrophoresis. Distinct protein bands were seen in gels stained with Coomassie brilliant blue after electrotransfer, implying that the blotting conditions left a considerable part of the OMV in the gels. This indicated that much less than 1 μg of the 140,000-, 86,000-, 65,000-, and 43,000-molecular-weight protein components was strongly antigenic with a

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1:200 dilution of several postvaccination sera. The class 2 protein of 41,000 molecular weight, which is the serotype protein of the meningococcal strain M986 (4), showed only weak antibody binding after vaccination. The antigenicity of this protein is readily lost upon denaturation to the monomeric form (16, 27) but may be partly restored upon inclusion of dipolar ionic detergent during the blotting procedure (12). Therefore, the antibody response detected by the present immunoblotting method will probably underestimate the immunological importance of the class 2 protein and possibly other antigens in the vaccine.

Rosenqvist et al. (18) have previously measured the levels of the different immunoglobulin classes in the individual sera by an ELISA. The antigen was an OMV fraction from the same meningococcal strain as used for immunoblotting. It was of interest to see whether there was a correspondence between high antibody levels determined by ELISA and marked immunoreactive bands on blots for the individual sera. We found that prevaccination sera, which showed distinct binding of IgG, IgA, or IgM antibodies to blots, had significantly higher levels of the respective antibodies than those giving weaker bands. As described above, the majority of these sera were from meningococcal carriers. This was in reasonable agreement with the ELISA observations showing that the levels before vaccination of IgG and IgA, but not IgM antibodies, were higher in meningococcal carriers than noncarriers (18).

The amount of IgA antibodies in the 6-week postvaccination sera which demonstrated distinct IgA-reactive bands on blots was also significantly higher than those yielding weak bands. Such a difference was not observed for the IgM antibodies binding to various OMV components or for the IgG antibodies that bound strongly to the 43,000-, 30,000-, or 22,000-molecular-weight antigens. The majority of the postvaccination sera that showed IgM antibody activity on blots were from carriers, whereas such correspondence was not found for the IgA antibodies. The ELISA measurements did not reveal any difference in the levels of the various antibody classes between carriers and noncarriers 6 weeks after vaccination (18). Thus, the positive relationship observed before vaccination between high antibody levels and distinct immunoreactive bands for all antibody classes only held for the IgA antibodies after vaccination.

## ACKNOWLEDGMENTS

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