INFECTION AND IMMUNITY, May 1989, p. 1590–1598
0019-9567/89/051590-09$02.00/0

Vol. 57, No. 5

Human Immune Response to Meningococcal Outer Membrane Protein Epitopes after Natural Infection or Vaccination

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Received 31 October 1988/Accepted 22 January 1989

Antibody levels in 41 sets of human acute- and convalescent-phase meningococcal sera were compared with those in 23 sets of human prevaccination and 2-week postvaccination sera. We used a modification of a solid-phase radioimmunoassay (SPRIA) technique to test each of the human serum samples as inhibitors of monoclonal antibodies (MAbs) that bind (HIMSPRIA) to the outer membrane complex from a 2a:P1.2:P5.1 strain. We used three murine MAbs specific for the 2a, P1.2, and P5.1 epitopes on meningococcal class 1, 2, and 5 proteins, respectively, to detect antibodies with similar specificities in human sera. Each of 40 available matching strains from patients were also screened with the three MAbs in a nitrocellulose spot blot assay. A total of 37 (92%) were positive for the 2a epitope, 36 (90%) were positive for the P1.2 epitope, and 16 (40%) were positive for the P5.1 epitope. Of 38 available convalescent-phase sera, 27 (71%) matched with these strains and had detectable inhibiting antibody for each of the MAb-defined protein epitopes of the infecting strain. Three convalescent-phase sera had no HIMSPRIA activity for MAb-defined epitopes that were present on the infecting strain; others had activity for one or two of the epitopes. The results were similar for pre- and postvaccination sera. The average level of HIMSPRIA activity for the P1.2 epitope was greater than fivefold higher in postvaccination sera compared with that in convalescent-phase sera. Sera with distinct patterns of HIMSPRIA activity also were tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis and showed a correlation between the HIMSPRIA activity for particular epitopes and the level of antibody binding to the immunoblotted proteins possessing those epitopes. A comparison of the HIMSPRIA and the bactericidal activity of selected postvaccination sera indicated a possible correlation between HIMSPRIA and bactericidal activity, but it also suggested the presence of bactericidal antibodies with specificities other than those defined by the MAbs.

Group B meningococcal disease continues to be a problem in various parts of the world (2, 3, 8, 15, 30, 34). The nonimmunogenicity of the purified group B polysaccharide in humans (46), the low avidity of anti-group B polysaccharide antibodies stimulated by natural infection or immunization (22, 53) and their lack of lytic activity with human complement (50), and concerns that the group B polysaccharide resembles structures that are present on human brain neural cell adhesion glycoproteins (12, 36) have resulted in a greater emphasis on the use of alternative meningococcal antigens for vaccines to prevent disease caused by group B meningococci.

The most recent meningococcal vaccines have been composed of a complex of both polysaccharides and outer membrane proteins (4, 24, 28, 44, 52, 53). Information regarding which outer membrane proteins are most important in the pathogenic mechanisms of meningococcal infection and the immunogenicity of these proteins in natural infections is necessary for the preparation of effective vaccines. Anti-protein antibodies in sera from humans with natural infections would reflect the immunogenicity of protein antigens that are in their native form. Anti-protein antibodies in postvaccination sera from humans reflect the immunogenicity of the proteins after they have been extracted from lipopolysaccharide and other outer membrane components. One method that has been useful for the assessment of the human antibody response to meningococcal proteins has been to separate proteins by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and then to test the separated proteins for binding by human sera (33, 45). This allows a qualitative assessment of the levels of antibody to the total array of vaccine proteins. One limitation of this technique is that the proteins must first be treated by conditions (2% SDS, 100°C) that have been shown to denature some of the important outer membrane proteins (23), especially the meningococcal class 2 and class 3 proteins (porins).

We wanted to quantify protein-specific antibodies in human sera by using nondegraded outer membrane proteins as antigens and to attempt to relate antibody specificity to the bactericidal activity of sera. Assays based on the competitive binding of monoclonal antibodies (MAbs) and polyclonal antibodies to native protein antigens have been described in studies in which the fine structure of viral outer coat protein epitopes has been mapped (41); in which human antibody specific for gonococcal protein I (38), gonococcal and meningococcal H.8 (5), and a neisserial 70-kilodalton protein (1) have been detected; and in which human antibodies specific for a gonococcal lipooligosaccharide epitope associated with serum resistance have been measured (20, 40). In a similar assay we used mouse MAbs specific for three different meningococcal outer membrane protein epitopes (54) to measure the immunogenicity of meningococcal proteins in humans who were vaccinated with a polysaccharide-protein complex or who had a meningococcal infection.

(Preliminary results of this study have been presented previously [R. E. Mandrell and W. D. Zollinger, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 615, 1983].)

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MATERIALS AND METHODS

Bacterial strains. Strains B16B6 and H355 were generously provided by Carl Frasch (Bethesda, Md.); strain 3006 was generously provided by Jan Poolman (Bilthoven, The Netherlands). All other strains were from the culture collection of the Department of Bacterial Diseases, Walter Reed Army Institute of Research (Washington, D.C.). Cultures were either lyophilized and sealed under vacuum or were frozen at −70°C. Included in the study were 40 strains of meningococci that were obtained between February 1965 and January 1969 from military recruits or military dependents with systemic meningococcal disease. One strain was from Fort Leonard Wood, Mo., one was from Fort Knox, Ky., and the remainder were from Fort Dix, N.J.

Human sera. Acute- and convalescent-phase serum pairs that were obtained from the same individuals as the strains described above (case strains) were used. The acute-phase serum was obtained a few days after the onset of disease and the convalescent-phase serum was obtained 10 to 32 days after the acute phase. The serum samples were stored at −20°C before use in this study. For comparison with the case sera and strains, we collected sera from 23 volunteers before and 2 weeks after their vaccination with a group B polysaccharide-protein complex vaccine (52) (vaccine BP2-5-5) made from a B:2a:P1.2:P5.1:L3,7 strain.

MAbs. The preparation and characterization of MAbs 3-1-P1.2, 1-1-P2a, and 1-1-P5.1 have been described previously (23, 54); each of the antibodies identified a separate epitope on meningococcal class 1, 2, or 5 proteins, respectively. MAb 3-1-P1.2 is of immunoglobulin class G2a (IgG2a), 1-1-P2a is IgG3, and 1-1-P5.1 is IgG (subclass was not determined). For this study the epitopes defined by the MAbs are referred to as the P1.2, 2a, and P5.1 epitopes, respectively.

The concentration of each MAb was determined by a modification (21) of a procedure described previously (48).

Nitrocellulose spot blot assay. A number of procedures have been described for serotyping meningococci directly on nitrocellulose (10, 11, 17, 54). Briefly, we grew each of the strains overnight on nutrient agar and then extracted some of the organisms with a cotton swab and gently applied them to a defined area of a templated piece of nitrocellulose paper. The sheets were air dried and then treated in succession with a blocking buffer (0.01 M Tris hydrochloride, 0.9% NaCl, 0.02% sodium azide, 2% casein [pH 7.4]), MAb ascites fluid diluted 1:500 in blocking buffer, and ^{125}I-labeled goat anti-mouse IgG in blocking buffer (54). After each of the antibody steps, the papers were washed with 0.01 M Tris hydrochloride–saline buffer (pH 7.4), twice with 0.01% Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.) in 0.01 M Tris hydrochloride–saline (pH 7.4), and once again with the first wash solution. The papers were dried and then used for autoradiography with X-Omat AR film (Eastman Kodak Co., Rochester, N.Y). Films were exposed at −70°C. Any spot darker than the background was designated as positive; usually, spots were clearly positive (dark) or negative (no exposure).

SDS-PAGE and immunoblot analysis. The outer membrane complex (OMC) of a C:2a:P1.2:P5.1:L3 strain and samples of BP2-5-5 vaccine (52) were separated by SDS-PAGE, and the separated proteins were then transferred to nitrocellulose for immunoblot analysis (7, 54). The nitrocellulose paper was then processed as described above (nitrocellulose spot blot assay), except that human serum samples were diluted 1:100 and bound human antibodies were detected with ^{125}I-labeled goat anti-human immunoglobulin (Kirkegaard and Perry, Gaithersburg, Md.).

Bactericidal assay. Each of the serum samples obtained before and 2 weeks after vaccination with BP2-5-5 were tested for the presence of bactericidal activity by using a previously described bactericidal assay (50). As a complement source we used fresh human serum that was preselected for the absence of lytic activity against the test strain but that had sufficient complement activity for support, in conjunction with antibody, for killing the test strain. Previous studies have shown that human anti-group B polysaccharide antibodies are markedly bactericidal when rabbit serum is used as the complement source, but not when human serum is used as the complement source (50).

HIMSPRIA. We used a modification of a previously described solid-phase radioimmunoassay (SPRIA) procedure (48) to measure, in human case and vaccinate sera, antibodies with specificities similar to those of mouse MAbs that recognize epitopes on meningococcal class 1, 2, and 5 proteins (23, 54). We used a meningococcal OMC preparation (53) from strain 138I (2a:P1.2:P5.1:L3) as the antigen; this strain possesses each of the epitopes defined by the three MAbs. To determine a limiting antigen concentration, each of the MAbs was first tested in a direct SPRIA with various concentrations of the OMC. We routinely used an antigen concentration of 1 to 5 μg/ml based on protein (19). Wells of a polyvinyl microtiter plate were sensitized with a limiting concentration of antigen diluted in Dulbecco phosphate-buffered saline (pH 7.4). The sensitized wells were incubated for 2 h at room temperature. Unbound antigen was removed, the wells were washed once with a protein filler (0.5% casein, 0.5% bovine serum albumin, and 0.1% sodium azide in Dulbecco phosphate-buffered saline), additional filler was added, and the wells were incubated for 1 h. The filler was removed and portions of twofold dilutions of human sera in filler were added and incubated with antigen for 1 h before the addition of an equal volume of an appropriate dilution of MAb (a dilution of MAb that resulted in 35 to 50% of the plateau binding level; approximately 1 μg/ml in filler). After the antibody mixture was incubated overnight, unbound antibodies were removed and the wells were washed once with filler and three times with phosphate-buffered saline. A total of 25 μl of ^{125}I-labeled goat anti-mouse IgG (Kirkegaard and Perry) containing 20 ng (approximately 2×10^4 to 4×10^4 cpm) of specific antibody diluted in filler was added and the wells were incubated for 4 to 6 h at room temperature. The unbound antibody was removed, and the wash steps were repeated as described above. The wells were cut and then counted in a gamma counter.

The human antibody inhibition of MAb SPRIA (HIMSPRIA) activity of sera is presented in two forms. One form is as the dilution of serum that inhibited 50% of the binding of the MAb to the OMC antigen. However, for comparison of the HIMSPRIA activity of vaccinates and case sera run in separate assays on separate days, the results are presented as units of antibody. For these assays a standard curve was constructed by using a human postvaccination serum sample that contained high levels of competitive antibody for each of the MAbs and that was arbitrarily assigned a value of 100 units of HIMSPRIA activity for each of the three MAb-defined epitopes. Study sera were assigned units of antibody value by using this standard curve.

The significance of differences between the HIMSPRIA activities of convalescent-phase sera corresponding to P5.1$^+$ and P5.1$^-$ strains was determined by an unpaired t test.

RESULTS

Nitrocellulose spot blotting of case strains with MAb. A group of 39 meningococcal case strains and 39 matching acute- and convalescent-phase sera (10 to 32 days after the acute infection) were gathered from the strain and serum collection of the Department of Bacterial Diseases. Each of the meningococcal case strains was tested in a nitrocellulose spot blotting assay for the presence of the MAb-defined epitopes, and the results are presented in Table 1. The majority of the group C strains possessed both the 2a and P1.2 epitopes that were defined by the MAbs. However, the P5.1 epitope was expressed on only 15 of 33 (45%) group C strains expressing both the 2a and P1.2 epitopes. There were three different epitope patterns for the group B strains included in the study. The results of the spot blot assay agreed with previous serotyping studies in which the serotype 2a determinant was identified on epidemic meningococcal case strains of this time period (32, 34).

HIMSPRIA activity in acute- and convalescent-phase sera. Forty-two sets of acute- and convalescent-phase sera and matching strains were screened by HIMSPRIA (Table 2). Only 40 matching strains were available: 36 group C and 4 group B. All of the sera were included in the initial HIMSPRIA, but these data are presented only in Table 2. A careful examination of the data in Table 2 indicated that (i) a majority of the acute-phase sera had no HIMSPRIA activity for any of the MAb-defined epitopes (exceptions were serum samples 3772 and 3809); (ii) the majority of the convalescent-phase sera exhibited an increase in activity compared with the acute-phase sera; certain convalescent-phase sera exhibited very high increases (>12-fold) in activity for single, or all three, epitopes (serum samples 3649, 3708, 3725, 3781, 3806, and 3808); (iii) a few convalescent-phase sera exhibited either an absence of activity or low activity for epitopes that were present on the homologous strain (serum samples 3700, 3713, 3732, 3744, 3759, 3762, 3765, 3368, 3816, 3818, and 3820). From these 42 serum samples, we selected 35 that had case strains positive for both the P1.2 and 2a MAbs. Only 16 of the selected 35 case strains were positive for the P5.1 MAb. We compared the HIMSPRIA activities of the acute- and convalescent-phase sera with those of 23 pairs of sera obtained before and 2 weeks after vaccination from volunteers who received a meningococcal group B polysaccharide-protein complex vaccine (52).

HIMSPRIA activity in sera of humans vaccinated with BP2-5-5. The geometric means of the units of HIMSPRIA

TABLE 1. Epitope patterns of case strains

Strain and no.	Epitope pattern[a]		
	1	2	5
Group C			
15	+	+	+
18	+	+	−
2	+	−	−
1	−	−	−
Group B			
1	+	+	+
1	+	+	−
1	−	+	−
1	−	−	−

[a] Indicates the presence or absence of the class 1, 2, or 5 protein epitopes; these epitopes are defined by MAbs 3-1-P1.2, 1-1-P2a, and 1-1-P5.1, respectively. Of the 40 strains tested, the number of strains with epitopes 1, 2, and 5 were 37, 36, and 16, respectively.

TABLE 2. Results of HIMSPRIA with acute- and convalescent-phase sera from patients with meningococcus infection

Serum[a]	Strain	Group	MAb binding pattern[b]			HIMSPRIA ($THIM_{50}$)[c]		
			1	2	5	1	2	5
3647	150I	C	+	+	−	1	2	1
3649	150I	C	+	+	−	380	6	1
3650	154I	C	−	−	−	1	1	1
3652	154I	C	−	−	−	1	2	1
3658	151I	C	+	+	+	1	1	1
3660	151I	C	+	+	+	3	4	2
3662	148I	C	+	+	+	1	1	1
3664	148I	C	+	+	+	3	12	10
3670	132I	C	+	+	+	1	1	1
3672	132I	C	+	+	+	2	10	14
3678	152I	C	+	+	−	1	1	1
3680	152I	C	+	+	−	9	16	1
3698	311I	C	+	+	+	1	1	1
3700	311I	C	+	+	+	1	7	1
3702	44I	C	+	+	−	1	2	1
3704	44I	C	+	+	−	3	3	1
3706	299I	C	+	+	+	1	2	1
3708	299I	C	+	+	+	6	24	167
3710	43II	B	+	+	−	1	1	1
3713	43II	B	+	+	−	1	2	1
3715	48I	B	+	+	+	1	2	1
3717	48I	B	+	+	+	3	14	27
3719	298I	B	−	−	−	1	1	1
3721	298I	B	−	−	−	1	1	1
3723	318I	C	+	+	+	1	1	1
3725	318I	C	+	+	+	6	14	44
3727	317I	C	+	+	−	1	1	1
3729	317I	C	+	+	−	5	3	1
3730	62I	C	+	−	−	1	1	1
3732	62I	C	+	−	−	1	1	1
3739	115I	C	+	+	−	1	1	1
3741	115I	C	+	+	−	10	12	1
3742	308I	C	+	+	−	1	1	1
3744	308I	C	+	+	−	1	9	1
3745	315I	C	+	+	−	1	1	1
3747	315I	C	+	+	−	3	6	1
3748	295I	C	+	+	−	1	3	1
3750	295I	C	+	+	−	7	21	1
3751	100I	C	+	+	+	1	1	1
3753	100I	C	+	+	+	5	5	4
3754	63V	B	−	+	−	1	1	1
3756	63V	B	−	+	−	1	2	1
3757	70I	C	+	−	−	1	3	1
3759	70I	C	+	−	−	1	3	1
3760	72I	C	+	+	−	1	1	1
3762	72I	C	+	+	−	1	1	1
3763	314I	C	+	+	−	1	1	1
3765	314I	C	+	+	−	1	1	1
3766	325I	C	+	+	−	1	1	1
3768	325I	C	+	+	−	1	3	1
3769	330I	C	+	+	+	1	2	1
3771	330I	C	+	+	+	8	22	36
3772	297I	C	+	+	−	4	1	1
3774	297I	C	+	+	−	4	11	2
3775	99I	C	+	+	−	1	2	1
3778	99I	C	+	+	−	2	9	1
3779	—[d]	—				1	2	1
3781	—	—				2	14	512
3782	322I	C	+	+	−	1	1	1
3784	322I	C	+	+	−	16	11	1
3788	309I	C	+	+	−	1	1	1
3790	309I	C	+	+	−	9	54	1
3791	104I	C	+	+	+	1	2	1
3793	104I	C	+	+	+	2	3	32

Continued on following page

TABLE 2—*Continued*

Serum[a]	Strain	Group	MAb binding pattern[b]			HIMSPRIA ($THIM_{50}$)[c]		
			1	2	5	1	2	5
3794	334I	C	+	+	−	1	1	1
3796	334I	C	+	+	−	11	5	1
3800	141I	C	+	+	+	1	1	1
3803	141I	C	+	+	+	16	10	3
3804	67I	C	+	+	+	1	1	1
3806	67I	C	+	+	+	45	27	58
3807	306I	C	+	+	−	1	1	1
3808	306I	C	+	+	−	5	44	1
3809	302I	C	+	+	+	5	6	1
3810	302I	C	+	+	+	10	8	21
3811	296I	C	+	+	−	1	2	1
3812	296I	C	+	+	−	23	24	4
3813	—	—				1	1	1
3814	—	—				3	7	1
3815	292I	C	+	+	+	1	1	1
3816	292I	C	+	+	+	2	2	1
3817	295I	C	+	+	+	1	1	1
3818	295I	C	+	+	+	10	2	1
3819	300I	C	+	+	+	1	1	1
3820	300I	C	+	+	+	4	12	1

[a] Serum samples are listed in pairs; the first serum sample was from the acute phase, and the second serum sample was from the convalescent phase, which was obtained 7 to 32 days after the acute-phase serum sample.

[b] Indicates the presence or absence of the MAb-defined epitope on the indicated class protein of the homologous strain.

[c] Indicates the level of human serum inhibition of the MAb binding to an epitope on the protein of the indicated class. Data are expressed as the reciprocal dilution of the titer of the human serum inhibiting 50% plateau binding of the MAb ($THIM_{50}$, sera inhibiting <50% at a 1:2 dilution were assigned a value of 1); the $THIM_{50}$ was determined by interpolation by using titers representing inhibition of >50 and <50%.

[d] —, No strain available.

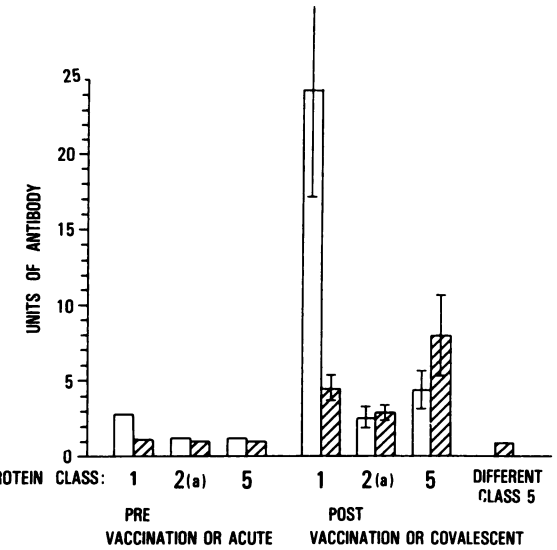

FIG. 1. Comparison of human antibody response to outer membrane proteins following vaccination or systemic infection. Human pre- and postvaccination sera (open bars) and acute- and convalescent-phase sera (hatched bars) were used as inhibitors of the binding of MAbs that identified the P1.2, 2a, or P5.1 epitopes of the class 1, 2, or 5 proteins, respectively (see text for details). The data are expressed as the geometric mean of the units of antibody determined for each serum sample. The units of antibody values were determined from a standard curve that used serum from a vaccinate that had high titers of inhibiting antibody for each of the MAb-defined epitopes; the serum was arbitrarily assigned a value of 100 units of antibody per ml. For this reason, only comparisons of the levels of antibody for a particular epitope, and between sera, should be made.

antibody specific for each MAb-defined epitope are presented in Fig. 1. The acute-phase sera had minimal HIMSPRIA activity for the P1.2, 2a, and P5.1 protein epitopes. The prevaccination sera were also negative, except for a few that contained a small amount of antibody for the class 1 epitope. (We emphasize that the units of antibody obtained with different MAbs cannot be compared directly since a different standard curve was used for each MAb.) The antibody specific for the P1.2 epitope was approximately five- to sixfold higher in the postvaccination sera (24.3 units) than in the convalescent-phase sera (4.5 units), whereas the antibody specific for the 2a or P5.1 epitopes differed by twofold or less (2a epitope, 2.4 to 2.8 units; P5.1 epitope, 4.3 to 8.0 units; postvaccination to convalescent phase). Of the 24 convalescent-phase sera corresponding to the $P5.1^-$ strains, only 1 exhibited any significant competition of the P5.1 MAb (Table 2, serum sample 3812), whereas the 16 $P5.1^+$ strains showed significant competition of the P5.1 MAb (convalescent-phase sera corresponding to $P5.1^-$ strains = 1.1 geometric mean units of the reciprocal of the titer of human serum inhibiting 50% plateau binding of the MAb [$THIM_{50}$] versus convalescent-phase sera corresponding to $P5.1^+$ strains = 8.6 geometric mean of $THIM_{50}$; $P \leq 0.0002$).

Seroconversion of patients and vaccinees with respect to HIMSPRIA activity. We compared the level of HIMSPRIA activity in each of the acute- and convalescent-phase sera and pre- and postvaccination sera sets for each of the epitopes. The data are presented in Fig. 2. The percentage of convalescent-phase sera with a twofold or greater rise in HIMSPRIA antibody to the three epitopes was similar: 76, 86, and 75% for the P1.2, 2a, and P5.1 epitopes, respectively (for the P5.1 epitope, only those sera matched with the $P5.1^+$ strains were included in the calculations). In comparison, the vaccination sera exibited 91, 44, and 57% seroconversion to the respective epitopes (data for individual sera are not shown).

Immunoblot analysis of selected area. To determine whether the HIMSPRIA activity of a serum sample correlated with its reactivity with the individual protein that carried the MAb-defined epitope, we selected a few serum samples with distinct patterns of HIMSPRIA antibody activity and tested them by immunoblot analysis. We tested a convalescent-phase and a postvaccination serum sample, each of which had elevated levels of competing antibody for each of the MAb-defined epitopes, and two pairs of pre- and postvaccination serum samples, one postvaccination serum sample without competing antibody for any epitope and one with competing antibody for the P5.1 epitope only. The results are shown in Fig. 3.

Antibody bound to the class 1, 2, and 5 proteins of both OMC and vaccine antigens and reflected the high level of HIMSPRIA activity for these epitopes. Lanes 7 to 10 of Fig. 3 show the results of two other pre- and postvaccination serum sample pairs that were negative by HIMSPRIA for the class 1 and 2 epitopes, but one postvaccination serum sample had 58 units of antibody specific for the class 5 epitope (Fig. 3, lane 10). Similarly, strong binding occurred only to the class 5 protein, as determined by immunoblot analysis. No binding to the class 1, 2, or 5 proteins occurred with pre- or postvaccination sera without HIMSPRIA activity for these epitopes (Fig. 3, lanes 7 and 8). However,

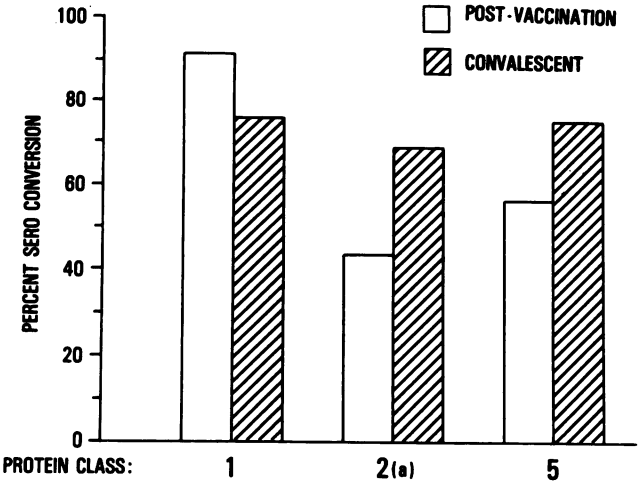

FIG. 2. Percentage of postvaccination or convalescent-phase sera with a twofold or greater increase in antibody against the indicated protein. For the P5.1 epitope, only those sera that were matched with the 16 P5.1$^+$ strains were included in the calculations.

binding to a high-molecular-weight protein (approx. 65,000; Fig. 3, lane 8) was noted with one of the serum samples.

Comparison of HIMSPRIA and bactericidal activity. We attempted to correlate the level of HIMSPRIA activity in a set of pre- and postvaccination sera with their level of antibody that was bactericidal for a series of group B strains with different protein and lipooligosaccharide (LOS) serotype patterns. The results are summarized in Table 3. Of 23 postvaccination sera, 4 had comparatively high levels of competitive antibody for the 2a epitope (data not shown), but they also had high levels of antibody for the P1.2 and P5.1 epitopes. In sera that had any activity, there was HIMSPRIA activity for more than one epitope, and this made it difficult to make comparisons of bactericidal (functional) and HIMSPRIA activity in order to determine whether there was bactericidal antibody specific for a particular epitope, protein class, or both. Therefore, six pairs of pre- and postvaccination sera that had relatively high levels

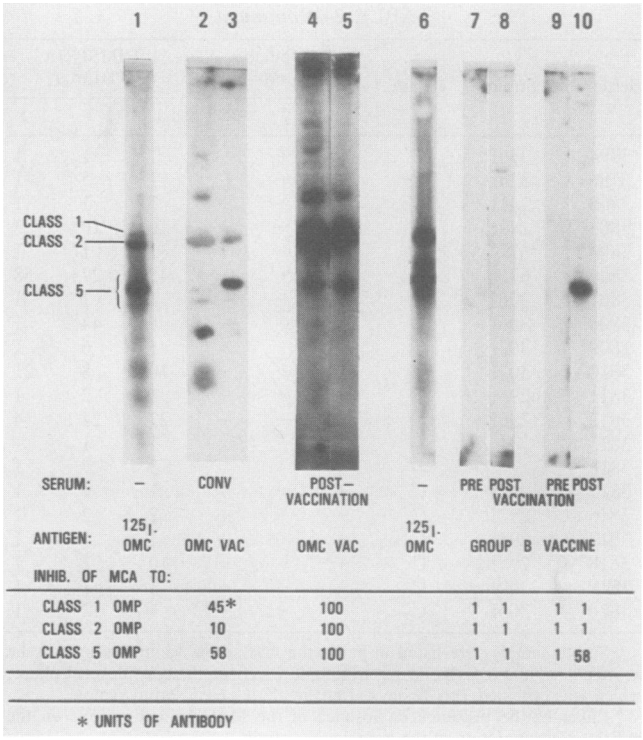

FIG. 3. Analysis of selected convalescent (CONV) and pre- and postvaccination sera by SDS-PAGE and immunoblot assay. The OMC of a C:2a:P1.2:P5.1:L3 strain and BP2-5-5 group B vaccine (VAC) were separated by SDS-PAGE, transferred to nitrocellulose, and tested with convalescent- or pre- or postvaccination sera. ^{125}I-labeled OMC was used as a control for the identification of the class 1, 2, and 5 proteins. The inhibitory activity of each serum sample is also listed, for comparison with the immunoblot activity. The serum in lanes 4 and 5 was used to construct the standard curve for the determination of units of inhibiting antibody. For further details of the procedure, see the text. Lanes 1 and 6, ^{125}I-labeled OMC of a C:2a:P1.2:P5.1:L3 strain; lanes 2 and 4, OMC of a C:2a:P1.2:P5.1:L3 strain; lanes 3, 5, and 7 to 10, BP2-5-5 group B vaccine. OMP, Outer membrane protein; MCA, monoclonal antibody.

TABLE 3. Comparison of HIMSPRIA and bactericidal activity in serum samples from selected individuals vaccinated with BP2-5-5

Serum	Weeks postvaccination	HIMSPRIA[a]			Bactericidal activity against the indicated strains[b]:					
		1	2	5	99M (+, +, +)	8047 (+, −, −)	3006 (+, −, −)	355 (−, −, −)	6155 (+, +, −)	B16B6 (+, +, −)
7570	0	1	1	1	1	1	64	1	2	32
7571	2	255	1	1	256	128	256	1	64	256
7582	0	3	1	1	1	1	1	1	256	16
7583	2	6	1	1	512	64	256	1	256	64
7594	0	1	1	1	1	1	1	1	64	16
7595	2	1	1	1	512	1	256	1	256	64
7778	0	2	1	1	4	4	>256	1	256	4
7779	2	25	2	1	512	16	>256	1	>256	64
7805	0	1	1	1	1	2	>256	1	>256	16
7806	2	1	1	10	512	4	>256	1	>256	64
7837	0	1	1	1	1	1	256	1	256	32
7838	2	29	2	2	128	16	>256	8	>256	>256

[a] HIMSPRIA activity is presented as the reciprocal of the highest titer of serum inhibiting 50% of plateau binding of the MAb that bound to the epitope on the indicated protein class.

[b] Data in parentheses provides a simplified designation of the MAb-binding profile for the protein class 1, 2, and 5 epitopes, respectively; a detailed serotype designation of each strain is as follows: 99M, B:2a:P1.2:P5.1:L3,7; 8047, B:2b;P1.2:L3,4,6; 3006, B:2b:P1.2:P5.2:L2,3; 355, B:15:P1.15:P5.2:L3,8; 6155, B:2a:P1.2:?:L3,7; B16B6, B:2a:P1.2:L2,(3). The BP2-5-5 vaccine was prepared from strain 99M; contamination by LOS was about 7%; bactericidal data are expressed as the reciprocal of the highest dilution of human serum that killed >50% of the organisms.

of HIMSPRIA antibody for only one of the three MAb-defined epitopes were selected for further study.

Five of six postvaccination sera had an increase in competitive antibody for at least one of the three epitopes; only the 7594-7595 serum sample pair had no increase in competitive antibody. Each of the postvaccination serum samples had a marked increase in bactericidal antibody for strain 99M (B:2a:P1.2:P5.1:L3,7), regardless of the level of competitive antibody; similarly, in each serum sample significant bactericidal activity for strains 3006 (B:2b:P1.2:P5.2:L2,3) and 6155 (B:2a:P1.2:?:L3,7) either was preexisting or increased by vaccination.

Strain 355 (B:15:P1.15:P5.2:L3,8), which shared only the L3 determinant with the vaccine strain (B:2a:P1.2:P5.2:L3,7), was lysed only by a relatively high concentration (≤1:8 dilution) of postvaccination serum. Serum sample 7595 had no HIMSPRIA antibody for the three MAb-defined epitopes, but it had appreciable levels of bactericidal antibody for each of the four strains (strains 99M, 3006, 6155, and B16B6) that shared at least one of the MAb-defined epitopes. Although each of the four strains shared P1.2 and L3 epitopes, strain 8047, which also shared these epitopes, and strain 355, which shared L3, were not lysed by serum sample 7595. Strain 355 was resistant to lysis by most of the human sera tested, including those that had significant levels of anti-P1.2 (Table 3, serum samples 7571, 7779, and 7838) or anti-P5.1 antibody (Table 3, serum sample 7806). Only one of the serum samples tested for bactericidal activity had appreciable levels of HIMSPRIA antibody for either the 2a or the P5.1 epitope (Table 3, serum sample 7806); yet all of the sera had appreciable bactericidal activities for some strains, and this appeared to be independent of the epitope profile of the strains.

Serum samples 7594 and 7595 had no HIMSPRIA activity for the three epitopes that were monitored in this study, and these results generally correlated with their lack of appreciable binding to proteins by immunoblot analysis (Fig. 3, lanes 7 and 8). The low HIMSPRIA activity and the absence of appreciable immunoblot activity in these serum samples did not correlate with their high level of bactericidal activity with certain strains. Close inspection of lane 8 of Fig. 3 revealed that serum sample 7595 bound to a protein with an M_r of approximately 65,000. Further investigation is needed to determine whether antibodies specific for this protein or to some other outer membrane structure not bound by antibody contributed to the bactericidal activity with strains 99M, 3006, 6155, and B16B6.

Of the six strains tested, strains 99M and 355 had serotype profiles that were the most and the least similar, respectively, to those of antigens in the vaccine. Similarly, each of the pairs of sera from the vaccinates that we tested exhibited increased bactericidal activity in the serum sample obtained 2 weeks postvaccination for strain 99M, but exhibited little or no bactericidal activity for strain 355.

DISCUSSION

We used three MAbs specific for separate epitopes on meningococcal class 1, 2, and 5 proteins to measure the level of antibody to these proteins in human pre- and postvaccination sera and sera from patients infected with strains having various combinations of the three MAb-defined epitopes.

A detailed analysis of the HIMSPRIA activity of sera from infected individuals (Table 2) and sera from vaccinates (data not shown) indicated that there is extensive heterogeneity in the human response to the three classes of proteins examined in this study. Some individuals had a high response to one protein epitope but little or no response to the other protein epitopes (Table 2), while other individuals had no measurable response to any of the protein epitopes (Table 1 and Fig. 2). A similar pattern of HIMSPRIA activity occurred with the postvaccination sera: 4 of 23 postvaccination serum samples had no HIMSPRIA activity for any of the three epitopes and 9 of 23 postvaccination serum samples had activity only for the P1.2 epitope (data not shown). The heterogeneous response to protein epitopes in vaccinated individuals was more surprising since, theoretically, they each received the same concentration of each of the proteins. The majority of individuals, however, had HIMSPRIA activity to at least one of the MAb-defined protein epitopes that was present on their infecting strain or in the vaccine. The HIMSPRIA activity of sera from vaccinates indicated a low seroconversion to the 2a and P5.1 epitopes compared with that to the P1.2 epitope: 44 and 57% of serum samples compared with 91% of serum samples, respectively, with a >twofold increase in activity ($THIM_{50}$). This may reflect a decreased immunogenicity of the class 2 and 5 proteins or simply a difference in the capacity to respond to these proteins because of the presence or absence of preexisting antibody. The fact that convalescent-phase sera exhibited higher rates of seroconversion to the 2a and P5.1 epitopes (Fig. 2) with equally low levels of preexisting antibody (Fig. 1) supports the former interpretation. However, the higher level of P1.2 HIMSPRIA activity in the prevaccination sera compared with that in the acute-phase sera (Fig. 1) reflected the higher activity for this epitope in postvaccination sera compared with that in convalescent-phase sera.

The differences in response to these protein epitopes can be compared with the results of other studies in which human sera and mouse monoclonal antibody inhibition assays were used to detect human antibody specific for meningococcal outer membrane proteins. A study by Black et al. (5) has shown that, of 13 patients with disseminated meningococcal and gonococcal infections, all had inhibiting antibody to the H.8 neisserial common antigen. In a separate study, an inhibition enzyme-linked immunosorbent assay was used to detect anti-70-kilodalton neisserial common protein antibodies that were present in each of the convalescent-phase serum samples of patients with meningococcal infection and most of the serum samples of carriers of meningococci (1). These results indicate that, during disseminated infections, humans respond to many different outer membrane proteins. Wedege and Frøholm (45) have also shown in an immunoblot assay that humans vaccinated with a meningococcal outer membrane protein-group B polysaccharide complex had a heterogeneous response to the individual proteins of the vaccine, but that the class 5 protein was the most immunogenic. The difference in class 5 protein immunogenicity measured in their study and our study could be due to differences between vaccine preparations, the number of vaccine doses, carrier status during the vaccine study, a variation in the human response to class 5 protein epitopes, and/or a decrease in the activity of class 1 and 2 proteins on SDS-PAGE.

The class 5 protein epitope (P5.1) exhibited a greater variance of expression compared with the class 1 and 2 protein epitopes, a characteristic of meningococcal class 5 proteins that has been noted by others (13, 31, 49). It is noteworthy that in our study, none of the convalescent-phase sera from patients infected with strains lacking the P5.1 epitope had significant HIMSPRIA activity for the P5.1 MAb. This indicates that after passage of the case strains on

artificial media, the presence or absence of the P5.1 epitope accurately reflected the status of the class 5 protein during infection. This is in contrast to the high variability of expression in humans of the analogous protein class of gonococci, PII (47).

The results of SDS-PAGE and immunoblot analysis with a few serum samples and strains generally agreed with the results of the HIMSPRIA analysis. Those sera with high levels of HIMSPRIA activity against protein classes 1, 2, or 5 also bound to those proteins on immunoblots (Fig. 3). However, differences in the concentration or expression of proteins (epitopes?) in different antigen preparations resulted in differences in serum antibody detection (Fig. 3, lanes 2 and 3; class 5). Although epitope density (concentration) could not be quantified accurately by the spot blot method, differences in the exposure intensity of the autoradiographic film could indicate that there are qualitative differences in epitope expression among strains. Variable epitope expression could explain some of the variation in antibody response. Other factors that could account for higher HIMSPRIA activity in some sera compared with that in others are (i) primed immune systems; (ii) persistence of infection or persistence of vaccine antigen; (iii) differences in the proportion of inhibiting (competitive) versus noninhibiting antibodies, including differences in immunoglobulin class; and (iv) antibody affinity. The last two factors are a concern in the interpretation of the results of any competitive binding assay.

The organization of the various classes of proteins that were present in the outer membrane is not clear. However, there is evidence of close associations between neisserial outer membrane antigens (18, 26, 29, 42, 43, 54). The similarities in the M_rs of the class 1 and 2 proteins (14) and the difficulty in separating these proteins with MAbs in immunoprecipitation studies (54; unpublished data) have suggested that a tight membrane association exists between these two proteins. We could not rule out the possibility that there may be steric interference between antibodies binding to the class 1 and 2 proteins, since only three case strains were available that had one epitope but not the other. However, two serum samples exhibited high levels of HIMSPRIA activity for either the class 1 or the class 2 protein epitope but not the other one (Table 2, serum samples 3649 and 3744), indicating that there was no steric hindrance in these instances. Also, the fact that convalescent-phase sera from patients infected with 2a:P1.2 strains that were P5.1$^-$ had high levels of P1.2 and 2a HIMSPRIA activity but no P5.1 HIMSPRIA activity (Table 2, serum samples 3680, 3741, 3750, and 3784) suggested that, although the class 1 and 2 proteins may have been associated, they were of sufficient spatial distance from the class 5 protein that binding of class 1- and class 2-specific human antibodies did not interfere with the binding of the class 5 protein MAb.

Since it has been shown that human anti-group B polysaccharide antibody is not bactericidal with human complement (50), the bactericidal activity measured in this study can be attributed to antibodies that are specific for outer membrane protein or LOS subcapsular antigens. We tested sera with distinct HIMSPRIA activities with MAb-defined strains in bactericidal assays to determine whether antibodies specific for a particular protein were bactericidal. However, a comparison of the patterns of HIMSPRIA and bactericidal activity in sera from human vaccinates did not reveal the specificity of the bactericidal antibody. Although there were sera that had high levels of HIMSPRIA activity to a single MAb-defined epitope and that were bactericidal for selected strains possessing those epitopes, there also were bactericidal sera without measurable HIMSPRIA activities. This may have been caused by the presence of antibodies specific for other epitopes on these or other proteins (1, 5, 27). Also, as noted in a previous study (9), some strains appeared to be more sensitive to serum than others. For example, strains 3006 and 6155 were killed even at relatively low concentrations of the prevaccination sera, while strain 355 was resistant (Table 3). The reasons for such differences are not clear, but they may be related to factors that are not related to antibody titers, such as the length of LOS oligosaccharides (16, 37), the amount and density of capsule (25), and the number or specificity of class 5 proteins that are expressed. These results indicate the difficulty in assigning the specificity of bactericidal antibodies.

To assign the bactericidal specificity to protein antigens probably requires purification of individual outer membrane proteins by nondegradative procedures, such as those used for the purification of gonococcal proteins (6, 35); the use of these proteins for the affinity purification of protein-specific human antibodies (35); and testing for their bactericidal activities. An alternative approach would be to test the functional activities of MAbs in bactericidal tests and animal protection models (39). However, the immunogenicity of meningococcal proteins in animals and their antigenicity with animal antibodies would not prove that the proteins have analogous activities in humans. Differences in the functional activities of human and animal antibodies induced by meningococcal proteins have been reported (51). Human anti-protein MAbs might provide a more relevant means for testing the functional activity of human antibodies to meningococcal proteins.

The inhibition of binding of mouse anti-protein MAbs by human polyclonal antibodies confirms the presence of human antibodies specific for identical or closely associated protein epitopes defined by MAbs. The inhibition assay provides an easy and fast method for analyzing the specificity of antibodies stimulated by neisserial outer membrane proteins.

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