Protection by Meningococcal Outer Membrane Protein PorA-Specific Antibodies and a Serogroup B Capsular Polysaccharide-Specific Antibody in Complement-Sufficient and C6-Deficient Infant Rats

Maija Toropainen,1* Leena Saarinen,1 Gestur Vidarsson,2,3 and Helena Käyhty1

National Public Health Institute, Department of Vaccines, Helsinki, Finland1; Immunotherapy Laboratory, Department of Immunology, University Medical Center Utrecht, Utrecht, The Netherlands2; and Laboratory of Vaccine Research, The Netherlands Vaccine Institute, Bilthoven, The Netherlands3

Received 4 November 2005/Returned for modification 9 December 2005/Accepted 9 February 2006

The relative contributions of antibody-induced complement-mediated bacterial lysis and antibody/complement-mediated phagocytosis to host immunity against meningococcal infections are currently unclear. Further, the in vivo effector functions of antibodies may vary depending on their specificity and Fc heavy-chain isotype. In this study, a mouse immunoglobulin G2a (mIgG2a) monoclonal antibody (MN12H2) to meningococcal outer membrane protein PorA (P1.16), its human IgG subclass derivatives (hIgG1 to hIgG4), and an mIgG2a monoclonal antibody (Nmb735) to serogroup B capsular polysaccharide (B-PS) were evaluated for passive protection against meningococcal serogroup B strain 44/76-SL (B:15:P1.7,16) in an infant rat infection model. Complement component C6-deficient (PVG/c) rats were used to assess the importance of complement-mediated bacterial lysis for protection. The PorA-specific parental mIgG2a and the hIgG1 to hIgG3 derivatives all induced efficient bactericidal activity in vitro in the presence of human or infant rat complement and augmented bacterial clearance in complement-sufficient HsdBr/BlHan:WIST rats, while the hIgG4 was unable to do so. In C6-deficient PVG/c rats, lacking complement-mediated bacterial lysis, the augmentation of bacterial clearance by PorA-specific mIgG2a and hIgG1 antibodies was impaired compared to that in the syngeneic complement-sufficient PVG/c rats strain. This was in contrast to the case for B-PS-specific mIgG2a, which conferred similar protective activity in both rat strains. These data suggest that while anti-B-PS antibody can provide protection in the infant rats without membrane attack complex formation, the protection afforded by anti-PorA antibody is more dependent on the activation of the whole complement pathway and subsequent bacterial lysis.

* Corresponding author. Mailing address: National Public Health Institute, Department of Vaccines, Vaccine Immunology Laboratory, Mannerheimintie 166, FIN-00300 Helsinki, Finland. Phone: 358-9-47448873. Fax: 358-9-47448599. E-mail: maija.toropainen@ktl.fi.

Meningitis and septicemia caused by Neisseria meningitidis (meningococcus) continue to cause morbidity and mortality worldwide. The important role of specific antibody and an intact complement system for protection against this pathogen is highlighted by the peak incidence of meningococcal infections in young children devoid of specific antibody (7), the inverse relation between the age-related decrease in the incidence of disease with the acquisition of serum bactericidal activity (BA) (7), and the increased frequency of systemic neisserial infections among persons with deficiencies in C3, alternative pathway, and especially late complement pathway components (C5 to C9) (4). Thus, BA, i.e., the ability of specific antibody to lyse bacterial cells in vitro in the presence of intact complement, has been considered crucial for evaluation of protection against meningococcal disease. Immunoglobulin G (IgG) antibodies to the outer membrane protein PorA, a major component of serogroup B outer membrane vesicle vaccine (5, 16) and an important antigen for meningococcal typing (1, 20), are frequently bactericidal and confer protection in an animal model of meningococcal infection (15, 18, 22). Good correlation between BA and protective activity in an infant rat model has been reported for mouse anti-PorA monoclonal antibodies (MAbs) (26). The protective activity of anti-PorA MAbs of human origin has not been measured in animal models of meningococcal infection. On the other hand, it has been recently shown that natural human antibodies to serogroup C capsular polysaccharide and serogroup B capsular polysaccharide (B-PS) can confer protection in vivo even in the absence of BA in vitro (27, 30).

Besides BA, several reports suggest that opsonophagocytic activity (OA) also is an important defense mechanism against meningococcal infections, especially those caused by serogroup B organisms (17, 19, 23). While BA is dependent on antibody-mediated deposition of the membrane attack complex on bacterial membranes through the activation of the whole complement cascade (C1 to C9), IgG-mediated phagocytosis is not. IgG-mediated phagocytosis is, however, amplified by complement activation but requires only deposition of opsonically active C3 split products (C3b and iC3b) on the bacterial surface. IgG and deposited C3 fragments can therefore function in concert as opsonins, targeting the invading pathogen for ingestion and killing by professional phagocytes through binding to Fcγ receptor (FcyR) and complement receptor. Increased OA has been shown in human sera taken at convalescence and after vaccination with serogroup B outer membrane vesicle vaccine (8, 9, 14, 24). These opsonic antibodies are directed to a variety of meningococcal surface antigens (13, 14), including the PorA protein. The relative importance of
To study the in vitro effector functions of anti-PorA antibodies in more detail, a panel of mouse-human chimeric MAbs of all the four human IgG subclasses (hlgG1 to hlgG4) with identical variable (V) genes against the P.1.16 epitope on PorA protein were generated from mouse IgG2a (mlgG2a) MAb MN12H2 (10) and characterized for their effector functions in vitro (29). While isotypes hlgG1 to hlgG3 mediated efficient bacterial lysis (relative activity, hlgG1 = hlgG3 > hlgG2) and phagocytosis (relative activity, hlgG3 > hlgG1 >> hlgG2), hlgG4 had undetectable activity in these assays. How these differences in functional activities in vitro are reflected in protection in vivo is not known.

In this study, the parental P.1.16 PorA-specific mlgG2a MAb MN12H2 (10), the hlgG1 to hlgG4 isotypes derived from it (29), and the B-PS-specific mlgG2a MAb Nmb735 (6) were assessed for protective activity in an infant rat infection model (21, 26). We had two major objectives: first, to assess the influence of antibody isotype on protection by comparing the protective activities of the P.1.16 PorA-specific hlgG1 to hlgG4 isotypes in complement sufficient animals, and second, to assess the importance of complement-mediated bacterial lysis for protection in vivo. This was done by comparing the protective activities of the B-PS specific mlgG2a antibody and the PorA-specific mlgG2a and hlgG1 antibodies in complement-sufficient and C6-deficient animals (12) with otherwise syngeneic backgrounds.


**RESULTS**

**Bactericidal activity.** The species of the complement source used in BA assays is known to affect the results greatly (34). Thus, the BAs of the MAbs were first reassessed using infant rat serum collected from 5- to 6-day-old HsdBrIHan:WIST rats or human serum as the exogenous complement source (Table 1). The results were similar to those obtained previously using human complement (29). Thus, B-PS-specific mouse MAb Nmb735 (not determined previously) and the parental PorA-specific mouse MAb MN12H2 (both IgG2a isotypes) were the most potent antibodies in this assay, followed by human PorA-specific IgG isotypes in the order of activity of hlgG1 = hlgG3 > hlgG2. The hlgG4 isotype failed to show any BA at the concentrations of up to 20 μg/ml tested (Table 1). Approximately threefold-higher antibody concentrations were needed for similar BAs (≥90% killing of the inoculum) when human complement was used, compared to rat, but this difference did not reach statistical significance (P = 0.06, paired one-sided t test).

**Effect of C6 deficiency on disease outcome in the infant rats.** Next, the protective activities of MAbs were assessed in an infant rat model of meningococcal infection. In these studies, one outbred rat strain (HsdBrIHan:WIST) and two syngeneic inbred rat strains, the complement-sufficient (PGV/c-) and the C6-deficient (PGV/c-) PVG strains, were used.

As the effect of C6 deficiency on the outcome of infection in nonimmune infant rats was not known, in preliminary studies
several challenge doses ($10^4$ to $10^6$ CFU/pup) of strain 44/76-SL were tested and the development of bacteremia (GM CFU per milliliter of blood) in PVG/c/H11002 rats compared to that in complement-sufficient HsdBrlHan:WIST rats. No significant differences between rat strains were found (Fig. 1). On the basis of these results, a dose of approximately $10^6$ CFU/pup, resulting in a GM blood bacterial density of approximately $10^6$ CFU/ml at 6 h postinfection, was chosen for protection studies with all rat strains.

Effect of antibody isotype on protection. To assess the influence of antibody isotype on protection, the protective activities of PorA-specific hlgG1 to hlgG4 isotypes in complement-sufficient HsdBrlHan:WIST rats were assessed (Fig. 2). The results resembled those obtained in the BA assay. Thus, hlgG1 and hlgG3 exhibited similar protective activities, while a four-fold-higher antibody dose (2 versus 0.5 µg/pup) of hlgG2 was needed for equal protective activity (Fig. 2). The nonbactericidal hlgG4 failed to show significant protective activity at the antibody doses of up to 20 µg/pup tested (Fig. 2).

Importance of complement-mediated bacterial lysis for protection. To assess the importance of antibody-induced complement-mediated bacterial lysis for protection, the B-PS-specific mlgG2a antibody Nmb735, the parental PorA-specific mlgG2a MN12H2, and the hlgG1 isotype derived from it were tested for protective activity in complement-sufficient (PVG/c+) and C6-deficient (PVG/c−) PVG rats of otherwise syngeneic background. For comparison, data obtained with the HsdBrlHan:WIST rat strain are included.

The B-PS-specific MAb Nmb735 was equally protective in all rat strains, with the lowest tested dose of 0.02 µg/pup conferring protection compared to control animals receiving saline (Fig. 3a). This was in contrast to PorA-specific MAb MN12H2, of which a 10-fold-higher antibody dose (5 versus 0.5 µg/pup) was needed for protection in the C6-deficient PVG/c− rats compared to complement-sufficient PVG/c+ or HsdBrlHan:WIST rats (Fig. 3b). The PorA-specific hlgG1 isotype failed to show any protective activity in PVG/c− rats at the doses up to 20 µg/pup tested (Fig. 3c). Similar results were obtained with the hlgG2 to hlgG4 isotypes (data not shown).

With Nmb735 and MN12H2, no significant differences in protective activity between the two complement sufficient rat strains were detected (Fig. 3a and b). This was in contrast to the hlgG1 isotype, of which a 10-fold-higher antibody dose (5 µg/pup) was needed for protection compared to control animals not given antibody (Fig. 3a). This was in contrast to the hlgG2 to hlgG4 isotypes (data not shown).

With Nmb735 and MN12H2, no significant differences in protective activity between the two complement sufficient rat strains were detected (Fig. 3a and b). This was in contrast to the hlgG1 isotype, of which a 10-fold-higher antibody dose (5 µg/pup) was needed for protection compared to control animals not given antibody (Fig. 3a). This was in contrast to the hlgG2 to hlgG4 isotypes (data not shown).

![FIG. 1. Development of bacteremia in complement-sufficient (Hsd BrlHan:WIST) and C6-deficient (PVG/c−) infant rats as a function of bacterial challenge dose. The results are given as GM bacteremia level (CFU per milliliter of blood) in each group of six animals. Error bars indicate standard deviations.](image1)

![FIG. 2. Protective activity of P1.16 PorA-specific hlgG1 to hlgG4 isotypes in complement-sufficient HsdBrlHan:WIST infant rats. The results are given as GM bacteremia level (CFU per milliliter of blood) in each group of five or six animals. Error bars indicate upper 95% confidence levels. The different isotypes were tested in separate experiments. *, P < 0.05 compared to control animals not given antibody, LSD. **, P value obtained with one-way ANOVA.](image2)
versus 0.5 μg/pup) was needed for equal protection in PVG/c+ compared to HsdBrlHan:WIST rats (Fig. 3c).

To study the possibility that rat strain-specific differences in complement activity might explain the lower protective activity of the hIgG1 isotype in PVG/c+ compared to HsdBrlHan:WIST rats, the in vitro BAs of the parental PorA-specific MAb MN12H2 and the hIgG1 isotype were reassessed using pooled sera from the rat strains as the exogenous complement source. No significant differences were detected between sera from the different strains (Fig. 4). As expected, the C6-deficient serum from PVG/c− rats failed to show any bactericidal activity up to 80% concentration (data not shown).

DISCUSSION

While there is evidence supporting the importance of both antibody-induced BA (4, 7) and OA (9, 19, 24) for protection against serogroup B meningococcal disease, their relative contributions to protective immunity have been difficult to delineate. The effector functions of antibodies may also vary depending on their specificity and Fc heavy-chain isotype. In this study, we addressed these questions by assessing the protective activity of PorA-specific and B-PS-specific mIgG2a antibodies in complement-sufficient and C6-deficient infant rats. Human IgG1 to IgG4 isotypes with identical V genes against the P1.16 epitope on the PorA outer membrane protein of N. meningitidis (29) were used to assess the influence of antibody isotype on protection. Our results showed that while the parental PorA-specific mIgG2a MAb MN12H2 and the human IgG1 to IgG3 isotypes derived from it efficiently enhanced bactericidal activity in vitro and augmented bacterial clearance in vivo in complement-sufficient rats, the augmentation of bacterial clearance by mIgG2a and hIgG1 antibodies was impaired in C6-defi-
cient animals. This was in contrast to a bactericidal, B-PS-specific mlgG2a MAb, Nmb735, which conferred similar protective activity in complement-sufficient and C6-deficient animals.

Similar to the case in our previous studies (25, 26) with an outbred HsdCpb:WU rat strain, the B-PS-specific mouse MAb Nmb735 and the parental PorA-specific mouse MAb MN12H2 efficiently induced BA in vitro and augmented bacterial clearance in vivo in both complement-sufficient HsdBrlHan:WIST and PVG/c+ rat strains. This was in contrast to the PorA-specific hlgG1 isotype, of which a 10-fold-higher antibody dose (5.0 versus 0.5 μg/pup) was needed for protection in PVG/c+ compared to HsdBrlHan:WIST animals. As no rat strain-specific differences in the BA in vitro were detected, the reason for the lower protective activity of this MAb in PVG/c+ animals remains to be fully evaluated. At least two possibilities exist to explain this difference between the BA in vitro and protection in vivo. First, the sensitivity of the BA assay (using 20% infant rat serum as an exogenous complement source) may not have been satisfactory to detect rat strain-specific differences in complement activity. Second, there may be strain-specific differences in the ability of rat phagocytes FcγR to bind antibody of human origin.

We found the relative protective activities of the PorA-specific MAbs to be mlgG2a = hlgG1 = hlgG3 > hlgG2 (hlgG4 had undetectable activity) in complement-sufficient HsdBrlHan:WIST rats and mlgG2a > hlgG1 in PVG/c+ rats. These results were consistent with the in vitro BA data in the presence of human complement (reference 29 and this study) or infant rat complement (this study), but the complete lack of protection by the hlgG1 isotype in C6-deficient PVG/c+ rats was somewhat at variance with the previous phagocytic activity data (29) obtained using human polymorphonuclear leukocytes as the effector cells and 44/76-SL bacteria as the target. A 10-fold-higher antibody dose of the parental mouse mlgG2a MAb MN12H2 was needed for protection in the C6-deficient PVG/c+ rat strain than in the isogenic complement-sufficient PVG/c+ rat strain. Possibly, an antibody dose higher than the 20 μg/pup tested would also have been needed for the hlgG1 isotype to confer protection in the PVG/c+ rat strain.

In contrast to the results with PorA-specific mlgG2a and hlgG1 MAbs, but similar to our previous studies with polyclonal, nonbactericidal B-PS-specific IgG antibodies of human origin (27), the B-PS-specific mlgG2a was equally protective in complement-sufficient and C6-deficient animals. These findings emphasize the importance of capsular polysaccharide-specific antibodies over subcapsular ones for protection in late complement-component-deficient individuals (2). Why antibodies to capsular compared to subcapsular antigen were more efficient in conferring protection in C6-deficient animals (this study) and also opsonophagocytosis in vitro (2) is not clear but has been suggested to arise from to the more exposed nature of the polysaccharide capsule and anticapsular antibodies and/or C3 fragments deposited by it on the bacterial surface (2, 3), where they are readily recognized by phagocytic cell FcRs and/or complement receptors. Despite the apparent dominance of anti-PorA antibody-mediated protection through complement-mediated bacterial lysis, this does not seem to be a common feature of all subcapsular meningococcal antigens. It has recently been demonstrated that despite the absence of BA in vitro, antibodies to other subcapsular antigens, such as transferrin binding protein A (33), genome-derived neisserial antigen 2132 (31), and genome-derived neisserial antigen 1870 (32), are able to confer protection in animal models of meningococcal infection. For the last two antigens, antibody-induced deposition of C3 fragments (C3b and iC3b) on the bacterial surface predicted well their ability to confer protection in vivo (31, 32), suggesting that OA was responsible for bacterial clearance.

To conclude, we have shown that while B-PS-specific antibody can confer protection in the infant rat without membrane attack complex formation, the protection afforded by PorA antibody is more dependent on the activation of the whole complement pathway and subsequent bacterial lysis. The relevance of these findings to protection in humans, and thus the validity of the infant rat model to assess the protective activity of antibodies of human origin in general, remain open until the rat homologs of human FcRs and their ligand specificities have been fully characterized. Furthermore, taking into consideration the importance of complement system for innate and specific immunity against meningococcal disease, a careful comparison of the rat and human complement systems would be needed to assess the validity of this infection model in general.

ACKNOWLEDGMENTS

We thank M. R. Daha for kindly providing the C6-deficient rat strain, H.-P. Harthus for providing Nmb735, B. Kuipers for providing MN12H2, H. Jarva for performing complement assays, L. Pyhäla for providing facilities for experimental work with animals, and the staff at the animal facilities of the National Public Health Institute in Helsinki and Kuopio, Finland, for comprehensive care of the animals. This work was funded partially by the World Health Organization, Global Program for Vaccines and Immunization, Vaccine Research and Development (contracts V23/181/74, V23/181/118), and the National Meningitis Trust, United Kingdom.

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Editor: J. N. Weiser