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Protective Activity of Monoclonal Antibodies to Genome-Derived Neisserial Antigen 1870, a *Neisseria meningitidis* Candidate Vaccine[1]

Jo Anne Welsch,* Raffaella Rossi,* Maurizio Comanducci,[†] and Dan M. Granoff[2]*

Genome-derived neisserial Ag (GNA) 1870 is a meningococcal vaccine candidate that can be subdivided into three variants based on amino acid sequence variability. Variant group 1 accounts for ~60% of disease-producing group B isolates. The Ag went unrecognized until its discovery by genome mining because it is expressed in low copy number by most strains. To investigate the relationship between Ab binding to GNA1870 and complement-mediated protective functions, we prepared a panel of four murine IgG mAbs against rGNA1870 (variant 1) and evaluated their activity against nine genetically diverse encapsulated *Neisseria meningitidis* strains expressing subvariants of variant 1 GNA1870. Based on flow cytometry with live encapsulated bacteria, surface accessibility of the epitopes recognized by the mAbs appeared to be low in most strains. Yet mAb concentrations <1 to 5 μg/ml were sufficient to elicit bactericidal activity with human complement and/or activate C3b deposition on the bacterial surface. Certain combinations of mAbs were highly bactericidal against strains that were resistant to bactericidal activity of the respective individual mAbs. The mAbs conferred passive protection against bacteremia in infant rats challenged by strains resistant to bacteriolysis, and the protective activity paralleled the ability of the mAb to activate C3b deposition. Thus, despite low GNA1870 surface exposure, anti-GNA1870 variant 1 Abs are bactericidal and/or elicit C3b deposition and confer protection against bacteremia caused by encapsulated *N. meningitidis* strains expressing GNA1870 subvariant 1 proteins. The data support GNA1870 as a promising vaccine candidate for prevention of meningococcal group B disease caused by GNA1870 variant 1 strains. *The Journal of Immunology,* **2004, 172: 5606–5615.**

Capsular group B strains of *Neisseria meningitidis* currently account for approximately one-third of cases of meningococcal disease in the U.S. (1, 2), and up to 80% of cases in Europe (3). Group B strains can also cause epidemics such as experienced in New Zealand for more than a decade (4). There is therefore a need for a vaccine with the potential to elicit broadly protective immunity to prevent group B meningococcal disease (reviewed in Refs. 5 and 6).

Based on extensive experience with polysaccharide-protein conjugate vaccines, the most logical target for a group B meningococcal vaccine would be the capsular polysaccharide. The group B capsule, however, consists of $\alpha(2\rightarrow 8)$*N*-acetyl-neuraminic acid, which is abundant in fetal tissues (7, 8). The polysaccharide, therefore, is a poor immunogen (9), and has the potential to elicit autoreactive Abs (10) with accompanying safety issues that are difficult to resolve. Noncapsular Ags are capable of eliciting protective anti-meningococcal Abs (11–15), and avoid the risk of eliciting autoreactive Abs to the group B capsular polysaccharide. For example, outer membrane vesicle vaccines are safe and efficacious in humans for prevention of group B disease (reviewed in Ref. 5), but the bactericidal Abs tend to be strain specific and are directed predominantly at PorA (16). The utility of these vaccines, therefore, is limited by extensive antigenic diversity of the surface-accessible loops of PorA (17, 18).

Reverse vaccinology (19) has been used to identify new vaccine candidates for prevention of group B meningococcal disease. This approach begins with the genomic sequence instead of Ags identified directly from studies of the microbial cell envelope. Sequences of unassembled DNA fragments are analyzed using computer algorithms to identify open reading frames that encode potentially novel surface-exposed proteins. Using this approach, Pizza et al. (20) identified a large number of genes that were predicted to encode conserved and surface-exposed meningococcal proteins. These genes were cloned and expressed in *Escherichia coli*, and a total of 28 recombinant proteins, designated genome-derived neisserial Ags (GNA),[3] was found to elicit Abs in mice that bound to the bacterial surface and/or had *N. meningitidis* bactericidal activity (20). To date, the most promising GNA vaccine candidates include GNA33, a mimetic of a surface-exposed loop of PorA (21); NadA, a surface-exposed molecule that may play a role in adherence (22); and two lipoproteins of unknown function, GNA2132 (23) and the recently identified GNA1870 (24).

GNA1870 can be subdivided into three variant groups based on amino acid sequence variability (24). Polyclonal antisera raised in mice to recombinant proteins representative of each of the three variants were bactericidal against most strains that carried the respective variant, but showed minimal cross-reactivity against strains from heterologous GNA1870 variant groups (24). Thus, the

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[3] Abbreviations used in this paper: GNA, genome-derived neisserial Ag; BC_{50}, $bactericidal_{50}$.

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variant groups defined by amino acid sequence similarity correlated with different antigenic groups.

Most vaccine Ags discovered by conventional studies of the cell envelope are expressed in relatively high copy number by the bacteria or, in the case of the polysaccharide capsule, are abundant on the cell surface. For such Ags, there are sufficient surface-accessible epitopes for Ab binding and activation of complement-mediated bacteriolysis, which confer protection against developing meningococcal disease (25, 26). In contrast, until genome mining (20), Ags such as GNA2132 or GNA1870 went unrecognized in large part because they are expressed in relatively low copy number by the bacteria and represent only a small fraction of the total protein in the cell envelope. At present, it is unclear whether Abs to such minor surface Ags are effective in conferring protection against meningococcal disease.

To better understand the functional activity of anti-GNA1870 Abs, we prepared a panel of murine mAbs to variant 1 GNA1870 recombinant protein, and investigated the relationship between binding to the bacterial surface of different encapsulated *N. meningitidis* strains and activation of complement-mediated bacteriolysis. We also investigated the ability of the mAbs to activate deposition of C3b on the bacterial surface because our previous studies showed that Abs that bind to the bacterial surface and activate C3b deposition can confer passive protective activity against meningococcal bacteremia in the infant rat model in the absence of bactericidal activity (23).

Materials and Methods

Bacterial strains

Nine *N. meningitidis* strains (eight capsular group B and one capsular group C; Table I), each expressing GNA1870 variant 1 proteins, were chosen for detailed characterization of the reactivity of the anti-GNA1870 mAbs. These strains were collected over a period of 25 years from patients hospitalized in the United Kingdom, Norway, The Netherlands, New Zealand, Cuba, or the U.S., and belong to three genetic clusters (four ET5 cluster strains, two ET37 cluster strains, and three lineage 3 strains). Strains M6190, M1390, and M4105 are recent clinical isolates from the U.S. (provided by T. Popovic, Centers For Disease Control and Prevention, Atlanta, GA). Strain NZ98/254 (described in some articles as NZ394/98 (23, 24)) is a representative isolate from a group B meningococcal epidemic in New Zealand (4). Strain NZ98/254 and group C strain 4243 were selected, in part, because they give reliable bacteremia in the infant rat challenge model (23, 27, 28). Several other *N. meningitidis* group B strains were used as controls in different experiments. These included strains 2996, M986, and 961-5945, representative of GNA1870 variant 2 group, and strain M1239, representative of the variant 3 group. We also used a mutant strain, MC58Δ1870 (24), in which the GNA1870 gene was inactivated (a gift from J. Abu-Bobbie, Chiron Vaccines).

DNA preparation and sequencing

The DNA sequencing of GNA1870 genes of the nine strains was performed on PCR products corresponding to genes amplified by primers external to the coding sequence on the chromosomal meningococcal DNA, as previously described (24). Fragment sizes of the PCR products were confirmed on a 1% agarose gel and were further purified using a Qiagen QIAquick PCR Purification kit (Qiagen, Valencia, CA). The DNA was diluted to ~10 ng/μl and submitted for sequence analysis (ABI 377 Automatic Sequencer; Applied Biosystems, Foster City, CA).

GNA1870 cloning, expression, and purification of recombinant proteins

The GNA1870 genes from *N. meningitidis* strains MC58 (variant 1), 961/5945 (variant 2), and M1239 (variant 3) were amplified by PCR using forward and reverse primers designed to amplify the GNA1870 coding sequence devoid of the sequence coding for the putative leader peptide, as previously described (24). The PCR products were digested and cloned into the pET-21b$^+$ expression vector (Novagen, Madison, WI), and the recombinant plasmids were transformed into *E. coli* BL21 (DE3). After growth at 37°C in Luria broth containing imperilling, 100 μg/ml, expression of the recombinant proteins was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (Sigma-Aldrich, St. Louis, MO). After treatment of the cells, as previously described, the GNA1870-His protein was eluted from a metal-chelate affinity chromatography column with 250 mM imidazole (24).

Polyclonal anti-rGNA1870 antisera

A total of 15 μg of rHisTag-GNA1870 protein was used to immunize 4- to 6-wk-old female CD-1 mice (10 mice per group). Injections were given i.p. CFA was used for the first dose, and IFA for two subsequent booster doses was given at 3-wk intervals. As controls, a group of 10 mice was immunized with the three injections of the respective adjuvant without vaccine. The polyclonal antiserum consisted of pooled individual mouse sera obtained 3 wk after the third immunization.

Preparation of mAbs

Four- to 6-wk-old female CD1 mice (Charles River Breeding Laboratories, Raleigh, NC) were immunized with 10 μg of a recombinant form of the GNA1870 protein. Dose 1 was given with CFA, and dose 2 was given 3 wk later with IFA. Two weeks after dose 2, blood samples were obtained and sera from individual animals were assayed for complement-mediated bactericidal activity against group B strain MC58. The mouse with the highest titer was boosted i.p. with a dose of the recombinant protein given with PBS. Three days later, the animal was sacrificed and the spleen cells were fused with myeloma cells (P3X63-Ag8.653) at a ratio of 1 spleen cell to 1.7 myeloma cell. After 2-wk incubation in hypoxanthine/aminopterin/thymidine selective medium, hybridoma supernatants were screened for complement-mediated bactericidal activity using strain MC58 and rabbit complement (see below). Specificity of complement-mediated bactericidal activity was demonstrated by a lack of bactericidal activity against a mutant strain of MC58 in which the gene encoding GNA1870 had been inactivated (MC58ΔGNA1870). Hybridomas secreting GNA1870-specific bactericidal Ab were cloned by limiting dilution and then expanded and frozen for subsequent use in tissue culture.

Abs from four cell lines were characterized in detail. Specificity of GNA1870 binding was confirmed by Western blot of solubilized whole cells of MC58, which showed a single band with an apparent mobility identical with that of rGNA1870 and absence of Ab binding to MC58ΔGNA1870 cells (see *Results*). The subclasses of the mAbs were determined using an Ab capture ELISA (10) with HRP-conjugated polyclonal Ab specific for each of the mouse IgG subclasses, IgM, IgA, and κ and λ L chains (Southern Biotechnology Associates, Birmingham, AL).

Table I. *N. meningitidis expressing variant 1 GNA1870*

Strain (Reference)	Country of Origin	Year Isolated	Serologic Classification	ET Cluster (Sequence Type, ST)[a]
MC58 (35) (36)	U.K.	1985	B:15:P1.7, 16	ET5 complex (74)
MC58ΔGNA1870 (24)	U.K.	1985	B:15:P1.7, 16	ET5 complex (74)
H44/76 (37)	Norway	1976	B:15:P1.7, 16b	ET5 complex (32)
CU385 (38)	Cuba	1980	B:4:P1.19, 15	ET5 complex (33)[b]
BZ83 (37, 39)	The Netherlands	1984	B:15:P-	ET5 complex (32)
4243 (40)	U.S.	1994	C:2a:P1.5,2	ET37 complex (11)[b]
M6190	U.S.	1999	B:2a:P1.5,2	ET37 complex (1988)[b]
NZ98/254 (4)	New Zealand	1998	B:4:P1.4	Lineage 3 (42)
M1390	U.S.	1995	B:15:B1.7,4	Lineage 3 (41)[b]
M4105	U.S.	1996	B:4,7:B1.7,4	Lineage 3 (154)[b]

[a] ST typing was performed by multilocus sequencing, as described (www.mlst.net).
[b] Indicates ST results for strains sequence typed for this study.

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With the exception of JAR3, which was grown in the presence of 5% FCS, the other hybridoma cell lines were grown in serum-free medium (220509; BD Biosciences, Bedford, MA). The mAbs were harvested from tissue culture medium by ammonium sulfate precipitation (50% w/v). After exhaustive dialysis in PBS buffer, the concentrations of the Abs were measured by the capture ELISA procedure.

Binding of Abs to the surface of live encapsulated meningococci

The ability of anti-rGNA1870 Abs to bind to the surface of live *N. meningitidis* was determined by flow cytometric detection of indirect fluorescence assay, performed as described previously (10, 29). FITC-conjugated $F(ab')_2$ goat anti-mouse IgG (H + L) (Jackson ImmunoResearch Laboratories, West Grove, PA) was the reagent used for fluorescence labeling. Positive control Abs included SEAM 12 and 41 (10) and C2/706 (30), anti-polysaccharide mAbs specific for encapsulated group B and C strains, respectively. Also, in some experiments, as a positive control we used a anti-PorA mAb specific for P1.2 (21). The negative control consisted of different dilutions of serum pools from control mice immunized with adjuvant alone.

Activation of human complement deposition on the surface of live encapsulated meningococci

Anti-GNA1870 Ab-dependent deposition of C3b or iC3b on the bacterial surface of live *N. meningitidis* bacteria was determined by flow cytometry, performed as previously described (23). Complement deposition on the bacterial surface was detected with FITC-conjugated sheep anti-human C3c (BioDesign, Saco, ME), which reacts with both C3b and iC3b. The complement source was serum from a healthy adult with no detectable intrinsic bactericidal activity, and no detectable group B or C anticapsular Ab when tested by ELISA. In the absence of added Ab, this serum gave no detectable deposition of complement on the bacterial surface of different strains. Positive and negative controls were those described above for measurement of Ab binding by flow cytometry.

Complement-dependent bactericidal Ab activity

After overnight growth on chocolate agar, several colonies of *N. meningitidis* were inoculated into Mueller-Hinton broth (starting $A_{620\ nm}$ of ~0.1) and the test organism was grown for ~2 h to an $A_{620\ nm}$ of ~0.6. After washing the bacteria twice in Gey's buffer, ~300–400 CFU were added to the reaction mixture. The assays were performed with human complement (described above) and, in selected experiments, were also performed with rabbit complement (pooled infant rabbit serum obtained from Cedarlane Laboratories, Hornby, Ontario, Canada). The final reaction mixture of 60 μl contained 20% (v/v) complement, and serial 2-fold dilutions of test sera or mAbs diluted in Gey's buffer. CFU/ml in the reaction mixtures was determined after overnight growth on chocolate agar (Remel, Rancho Cordova, CA). Bactericidal titers or concentrations were defined as the serum dilution (or Ab concentration), resulting in a 50% decrease in CFU/ml after 60-min incubation of bacteria in the reaction mixture, compared with the control CFU/ml at time 0. Typically, bacteria incubated with the negative control Ab and complement showed a 150–200% increase in CFU/ml during the 60 min of incubation.

Passive protection in infant rats

The ability of the anti-GNA1870 Abs to confer passive protection against *N. meningitidis* bacteremia was tested in infant rats challenged i.p., performed as previously described (21, 23, 29). In brief, 5- to 7-day-old pups from litters of outbred Wistar rats (Charles River Breeding Laboratories) were randomly redistributed to the nursing mothers. Groups of 5–10 animals were treated i.p. at time 0 with different dilutions of test or control antisera or mAbs. Two hours later, the animals were challenged i.p. with 100 μl of washed, log-phase *N. meningitidis*. The group C strain 4243 challenge dose was ~1000 CFU/rat, and the group B strain NZ98/254 dose was ~10,000 CFU/rat. Eighteen hours after the bacterial challenge, blood specimens were obtained by puncturing the heart with a syringe and needle containing ~25 U of heparin without preservative (American Pharmaceutical Partners, Los Angeles, CA). Aliquots of 1, 10, and 100 μl of blood were plated onto chocolate agar. The CFU/ml blood was determined after overnight incubation of the plates at 37°C in 5% CO_2.

Solubilized bacterial cell preparations

Bacterial cells were grown at 37°C to mid-log phase in Mueller-Hinton broth supplemented with 0.25% (w/v) glucose. The cells were harvested by centrifugation, and resuspended in SDS sample buffer (0.06 M Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-ME, 10 μg/ml bromphenol blue). After three cycles of freezing on dry ice, thawing at room temperature, and vortexing, the samples were boiled at 100°C and loaded onto the 10% SDS-PAGE gel, as described below.

SDS-PAGE and Western blots

Membrane preparations were analyzed using 15% SDS-PAGE, as described by Laemmli (31) using a MiniProtean II electrophoresis apparatus (Bio-Rad, Richmond, CA). For Western blots, the gel was equilibrated with buffer (48 mM Tris-HCl, 39 mM glycine, pH 9.0, 20% (v/v) methanol) and transferred to a nitrocellulose membrane (Bio-Rad) using a Trans-Blot (Bio-Rad) semidry electrophoretic transfer cell. The nitrocellulose membranes were blocked with 2% (w/v) nonfat milk in PBS. Anti-rGNA1870 antiserum, or each of the JAR mAbs was diluted in PBS containing 1% (w/v) BSA and 1% (w/v) Tween 20. Bound Ab was detected using rabbit anti-mouse IgG + A + M-HRP-conjugated polyclonal Ab (Zymed Laboratories, South San Francisco, CA) and Western Lightning chemiluminescence reagents (PerkinElmer Life Sciences, Boston, MA).

Results

Hybridoma cell lines

From a single fusion of mouse spleen cells, we isolated 26 hybridoma cell lines producing Abs that were bactericidal against *N. meningitidis* strain MC58 when tested with rabbit complement. Rabbit complement was chosen for the screening procedure because, as compared with human complement, rabbit complement substantially augments meningococcal bactericidal titers (32, 33) and, thus, increased the sensitivity of the screening procedure. Six cell lines, designated JAR1 to JAR6, were cloned and expanded for further analysis. Because of low functional activity of mAbs secreted by JAR2 and JAR6, the results reported below are for four of the mAbs, designated JAR1 (IgG2), JAR3 (IgG3), JAR4 (IgG2a), and JAR5 (IgG2b).

Anti-GNA1870 mAb binding by ELISA

As described above, GNA1870 can be subdivided into three variant groups based on amino acid sequence variability. In a previous study, mouse polyclonal antisera raised to recombinant proteins representative of each of the three variants showed minimal cross-reactive bactericidal activity against strains from heterologous GNA1870 variant groups (24). Using rGNA1870 proteins expressed from the genes of *N. meningitidis* strains MC58 (variant 1), 2996 (variant 2), or M1239 (variant 3) as the antigenic targets, we determined cross-reactivity by ELISA of the four mAbs prepared against rGNA1870 expressed from the gene of MC58 (variant 1). With the variant 1 protein as the antigenic target, the concentrations of Ab that resulted in an ELISA $OD_{405} = 0.5$ were 0.003, 0.02, 0.003, and 0.001 μg/ml for JAR1, 3, 4, and 5, respectively. With variant 2 and 3 proteins as the antigenic targets, JAR1, 3, and 5 showed no reactivity (OD_{405} <0.05) when tested at mAb concentrations as high as 50 μg/ml. In contrast, JAR4 cross-reacted with the variant 2 recombinant protein (0.05 μg/ml of the mAb gave an OD = 0.5), and this mAb also gave a weak reaction with the variant 3 recombinant protein (50 μg/ml gave an OD = 0.16, 3-fold above background). Although JAR4 cross-reacted strongly by ELISA with the variant 2 rGNA1870 encoded by the gene from strain 2996, this mAb was completely negative for bactericidal activity, binding, or activation of C3b deposition on the surface live bacteria from strain 2996 or two other group B strains expressing GNA1870 variant 2 proteins (strains M986 and 8047) (data not shown). Therefore, the results described below are limited to *N. meningitidis* strains with subvariants of variant 1 GNA1870 proteins.

Anti-GNA1870 mAb bactericidal activity

As expected from the screening assay used to select the hybridoma cell lines, all four mAbs were bactericidal with rabbit complement

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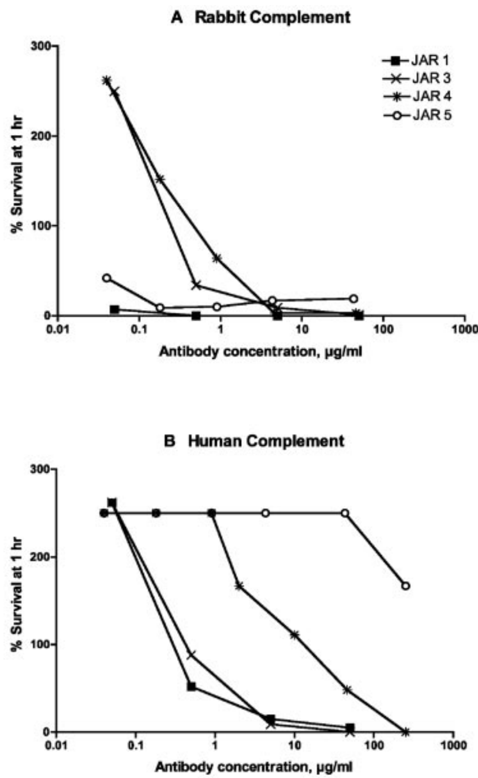

FIGURE 1. Survival of *N. meningitidis* group B strain MC58 after incubation for 60 min at 37°C in the presence of different concentrations (μg/ml) of JAR1, 3, 4, or 5 and 20% complement. *A*, Baby rabbit complement. *B*, Human complement. JAR1 (■), JAR3 (x), JAR4 (star), and JAR5 (○). Note that the mAbs were made against rGNA1870 encoded by the gene from MC58.

(Fig. 1*A*) when tested against group B strain MC58 (containing the GNA1870 gene used to express the recombinant protein for preparation of the mAbs). The bactericidal activity against MC58 was specific for GNA1870 because none of the mAbs was bactericidal against a mutant of MC58 in which the gene encoding GNA1870 had been inactivated (bactericidal $(BC)_{50}$ >50 μg/ml; Table II). When human serum was used as a complement source instead of rabbit complement (Fig. 1*B*), JAR1 and JAR3 showed strong bactericidal activity (BC_{50} <1 μg/ml) against the MC58 parent strain, JAR4 was bactericidal at concentrations ≥50 μg/ml, and JAR5 showed no significant activity. The lower bactericidal activity of some of the mAbs when measured with human complement was not unexpected because for reasons that are poorly understood, rabbit complement is known to augment meningococcal bactericidal titers substantially (32–34).

Table II summarizes the bactericidal activity of each of the mAbs when measured with human or rabbit complement against eight other GNA1870 variant 1 *N. meningitidis* strains. With one exception, bactericidal activity with human complement was observed only with the ET5 complex strains, all of which express GNA1870 proteins with amino acid sequences that are 100% identical with that of MC58 (legend to Fig. 2). The exception was JAR4, which was weakly bactericidal against strain 4243 (ET37 complex, BC_{50} ≥50 μg/ml). None of the other mAbs was bactericidal with human complement against *N. meningitidis* strains of ET37 complex or lineage 3, although as shown in Fig. 2, their respective GNA1870 amino acid sequences ranged from 92% identical (NZ98/254) as compared with that of MC58, to 96% identical (strains M4105 and 4243). The *gna1870* DNA sequences for the strains have been deposited in GenBank (accession numbers AY548370-AY548377). Inspection of the respective GNA1870 amino acid sequences revealed no obvious polymorphism that consistently predicted susceptibility or resistance of a strain to bactericidal activity of the different mAbs, although substitutions at amino acids located at positions 166 to 169, 197, or 217 to 224 are possibilities (see Fig. 2).

Certain mAbs that failed to kill individually, or showed minimal bactericidal activity, could in combination elicit strong bactericidal activity. Several examples are shown in Table III. Survival of strain 4243 after 60-min incubation with 50 μg/ml JAR3 or JAR4 and human complement was 106 and 85%, respectively, as compared with the respective CFU at time 0 for the negative control. The survival of strain NZ98/254 with 50 μg/ml JAR3 or JAR4 was 229 and 260%, respectively. In contrast, a combination of JAR3 and JAR4 and human complement killed each of these strains at concentrations as low as 2–10 μg/ml. In other experiments not shown, there was no significant synergy in bactericidal activity of combinations of JAR1 and JAR3, but there was synergistic bactericidal activity with a combination of JAR4 and JAR5 and human complement against strains 4243, NZ98/254, and MC58.

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Table II. *Bactericidal activity by anti-GNA1870 Abs*

Strain	Electrophoretic Type Cluster	Mouse Polyclonal Anti-GNA1870 1/BC Titer[a]		mAb JAR1 IgG3 BC_{50} (μg/ml)[b]		mAb JAR3 IgG3 BC_{50} (μg/ml)[b]		mAb JAR4 IgG2a BC_{50} (μg/ml)[b]		mAb JAR5 IgG2b BC_{50} (μg/ml)[b]	
		Hu C	Rab C	Hu C	Rab C	Hu C	Rab C	Hu C	Rab C	Hu C	Rab C
MC58[c]	ET5 complex	5,000	>10,000	0.5	<0.05	1.0	0.25	50	1	>50	0.03
MC58 GNA1870KO	ET5 complex	<10	<10	>50	>50	>50	>50	>50	>50	>50	>50
H44/76	ET5 complex	1,000	10,000	0.6	0.5	4.0	7.0	>50	0.5	>50	0.03
Cu385	ET5 complex	2,500	16,000	1.3	0.1	0.5	<0.05	>50	1	>50	0.1
BZ83	ET5 complex	2,000	2,500	0.3	<0.2	0.2	<0.2	50	9	>50	0.3
4243	ET37 complex	300	2,000	>50	>50	>50	>50	≥50	≥50	>50	>50
M6190	ET37 complex	<10	<10	>50	>50	>50	>50	>50	>50	>50	>50
NZ98/254	Lineage 3	<10	<10	>50	>50	>50	>50	>50	7	>50	>50
M1390	Lineage 3	<10	<10	>50	>50	>50	>50	>50	6	>50	0.4
M4105	Lineage 3	<10	<10	>50	>50	>50	>50	>50	>50	>50	>50

[a] BC titer is the dilution of serum giving 50% decrease in CFU/ml after 1-h incubation with complement as compared with CFU of controls at time 0. Data are shown for human (Hu) and rabbit (Rab) complement (C). (See examples in Fig. 1).

[b] BC_{50} is the concentration of mAb (μg/ml) that yields a 50% decrease in CFU/ml after 1-h incubation with bacteria and complement as compared with that of controls at time 0.

[c] Source for DNA sequence used to express rGNA1870 (see *Materials and Methods*).

```
MC58      01:VNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDH: 45
4243      01:---------------------------------------------: 45
M6190     01:----T-------A--------------------------------: 45
NZ394/98  01:--------F---A--------------------------------: 45
M4105     01:------------A--------------------------------: 45
2996      01:------------A--------------------------------: 45

MC58      46:KDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRF: 95
4243      46:--------M-----------------------------------------: 95
M6190     46:--------------------------------------------------: 95
NZ394/98  46:---S----------------------------------------------: 95
M4105     46:---S----------------------------------------------: 95
2996      46:---S----------------------------------------------: 95

MC58      96:DFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAK: 145
4243      96:--------------------------------L----V----D-------: 145
M6190     96:--------N-----------------------L----V------R-----: 145
NZ394/98  96:--------------------------------L----E--P---------: 145
M4105     96:--------------------------------L----V------------: 145
2996      96:---------------------I---D---VV-L-I-K-NNPDKIDSLINQ: 145

MC58     146:R     QFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQ: 195
4243     146:-     ------------------KDVM---------------------------: 195
M6190    146:-     ------------------K-DS---------------------------: 195
NZ394/98 146:-     R-K---------------KDVM---------------------------: 195
M4105    146:-     -----------------------------------S-------------: 195
2996     146:HGKIERS-LVSGLG----A-NQ--D- K-E-H-K--S------------------: 199

MC58     196:GNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGI: 245
4243     196:-H------------------Y----E-H----------------------: 245
M6190    196:-Y------------------Y----E-H------------D---------: 245
NZ394/98 196:-H-------------E--T-Y----E-H------------D---------: 245
M4105    196:-H-----------------S-----K------------------------: 245
2996     200:-     ---T--Q--E----EL-A-E-S----L-DTR-GSE---T-H-AL: 245

MC58     246:FGGKAQEVAGSAEVKTVNGIRHIGLAAKQ: 274
4243     246:-----------------------------: 274
M6190    246:---Q------------A------------: 274
NZ394/98 246:---Q----------E-A---H--------: 274
M4105    246:---Q----------E-A------------: 274
2996     246:--DR---I----T--IGEKVHE--I-G--: 274
```

FIGURE 2. Predicted amino acid sequences of GNA1870 of *N. meningitidis* test strains (Table II). The amino acid sequence for GNA1870 from MC58 is shown as representative of the gene for the other ET5 strains because the amino acid sequences for all four ET5 strains are identical. NZ98/254 and M1390 amino acid sequences are identical, and therefore NZ98/254 is depicted as representative of the sequences from these two strains. The sequence of strain 2996 is shown as representative of GNA1870 variant 2.

When each of these mAbs was tested individually, these strains were resistant to bactericidal activity with human complement (Table II).

Table III also shows data on the bactericidal activity of JAR3 and JAR4 tested against strain NZ98/254 with rabbit complement. Although the polyclonal anti-GNA1870 antiserum was negative (titer <1:16), the BC_{50} of JAR4 was ~3.5 μg/ml, and that of JAR3 was >50 μg/ml. The BC_{50} of a combination of JAR3 and JAR4 (0.18 μg/ml) was 20-fold lower than that of JAR4 when tested alone and >250 lower than that of JAR3. In another experiment with strain NZ98/254 not shown, a combination of JAR4 and JAR5 was bactericidal with rabbit complement at a mAb concentration of <0.15 μg/ml, although when JAR5 was tested alone it was not bactericidal (BC_{50} >50 μg/ml).

Expression of GNA1870 by N. meningitidis *strains*

In a previous study, *N. meningitidis* strains were subdivided based on low, intermediate, or high expression of the GNA1870 protein as measured by Western blot, and strains with low expression were found to be less susceptible to anti-GNA1870 bactericidal activity than strains with high expression (24). As described in *Materials and Methods*, to estimate GNA1870 expression we solubilized cells from strain MC58 and the MC58ΔGNA1870 mutant, and from three of the *N. meningitidis* strains resistant to bacteriolysis, 4243 (ET37 complex), M6190 (ET37 complex), and NZ98/254 (lineage 3). Equal amounts of protein from each of the strains were applied to the lanes, and the respective proteins were separated by SDS-PAGE. By Coomassie staining, a band corresponding to purified rGNA1870 (detected at 1 μg, but not 0.1 μg) was visible, but bands corresponding to GNA1870 were not visible in the lanes containing solubilized *N. meningitidis* cells (data not shown). By Western blot, GNA1870 contained in the solubilized cells was detected using polyclonal anti-rGNA1870 antiserum (Fig. 3*A*). With the exception of the MC58ΔGNA1870, which was negative, the bands corresponding to GNA1870 in the other lanes showed approximately similar reactivity to each other. Thus, lack of anti-GNA1870 bactericidal activity against some strains does not appear to correlate with lower expression of GNA1870. The polyclonal anti-GNA1870 antiserum reacted by Western blot with a second band of unknown identity with an apparent molecular mass

Table III. *Combinations of JAR3 and JAR4 enhance bactericidal activity against* N. meningitidis *strains 4243 and NZ98/254*

	Human Complement					Rabbit Complement		
	Strain 4243			Strain NZ98/254		Strain NZ98/254		
Antisera of mAb	Dilution or μg/ml	CFU/12 μl[a]	Percentage of Survival[b]	CFU/12 μl[a]	Percentage of Survival[b]	Dilution or μg/ml	CFU/12 μl[a]	Percentage of Survival[b]
Polyclonal anti-GNA1870	1/10	5	10	189	197	1:16	≥250	≥263
	1/50	1	2	≥250	260	1:64	≥250	≥263
	1/250	1	2	ND[c]	ND	1:256[d]	≥250	≥263
Negative control serum	1/10	140	292	≥250	260	1:16	≥250	≥263
	1/100	140	292	ND	ND	1:32	≥250	≥263
JAR3	50	51	106	220	229	50	≥250	≥263
	10	61	127	231	241	10	≥250	≥263
	2	72	150	244	254	2	≥250	≥263
JAR4	50	41	85	≥250	≥260	10	6	6
	10	123	250	≥250	≥260	5	8	8
	2	140	292	≥250	≥260	2.5	71	75
						1.25	200	211
JAR3 + JAR4	50	0	0	1	1	5	1	1
	10	0	0	11	11	1.25	0	0
	2	14	29	106	110	0.3	19	20
	0.4	59	123	≥250	≥260	0.15	54	57

[a] CFUs in 12 μl of the 60-μl reaction mixture after 1-h incubation with mAb and human complement.
[b] Percentage of survival is calculated based on CFU/12 μl present after 1-h incubation with complement and Ab as compared with that of the average CFU/12 μl in negative control vials measured at time 0. In the absence of Ab or complement, CFU/12 μl typically increase by 150–250% during the incubation.
[c] Not done.
[d] In other experiments not shown, there was no bactericidal activity at dilutions as high as 1/6250.

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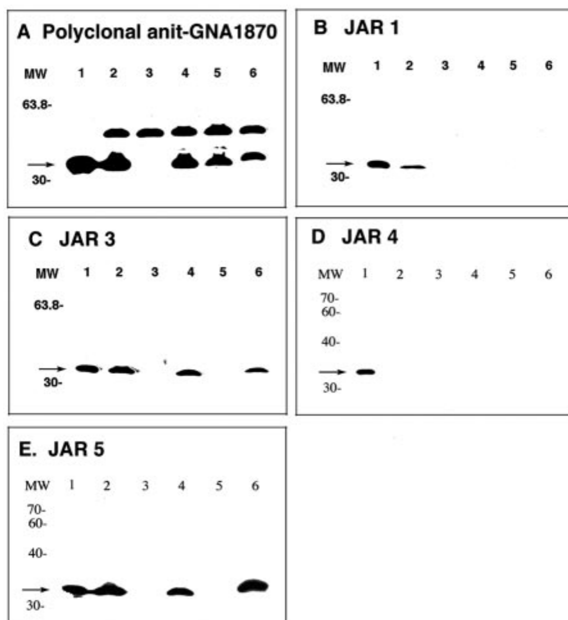

FIGURE 3. Western blot of solubilized whole cells from *N. meningitidis* strains. The blot was developed with either the anti-rGNA1870 polyclonal antiserum (*A*), or mAbs JAR1 (*B*), JAR3 (*C*), JAR4 (*D*), or JAR5 (*E*). rGNA1870 (0.1 μg) from MC58 (*lane 1*); solubilized cells from strains MC58 (*lane 2*); MC58ΔGNA1870 (*lane 3*); 4243 (*lane 4*); M6190 (*lane 5*); and NZ98/254 (*lane 6*). GNA1870 protein migrates with a molecular mass of ~29 kDa (arrow). With the polyclonal antiserum (*A*), there is an unidentified band of ~37 kDa expressed by every strain including the knockout. rGNA1870 polyclonal antiserum was used at a 1/10,000 dilution; the JAR mAbs were each used at a concentration of 0.5 μg/ml.

of ~37 kDa. This band was present in every strain tested including the GNA1870 knockout (*lane 3*). The 37-kDa Ag does not appear to be surface accessible nor to contribute to anti-GNA1870 bactericidal activity because the polyclonal antiserum showed no binding to the bacterial surface of the mutant MC58ΔGNA1870 strain as measured by flow cytometry (see below), and had no bactericidal activity against the mutant (titer <1:10 with human or rabbit complement; Table II).

Fig. 3, *B–E*, shows the corresponding reactivity of each of the four anti-GNA1870 mAbs in a Western blot. JAR1 (Fig. 3*B*) appeared to react with an epitope that is expressed only by GNA1870 from the ET5 complex strain MC58, while JAR3 (Fig. 3*C*) and JAR5 (Fig. 3*E*) detected epitopes shared by GNA1870 expressed by strain MC58 and some ET37 complex (4343, but not M6190 (*lanes 4* and *5*, respectively)) and lineage 3 strains (NZ98/254 (*lane 6*)). JAR4 (Fig. 3*D*), which was negative on the Western blot with any of the strains, may detect a conformational epitope that is poorly expressed by solubilized *N. meningitidis* cell under the denaturing conditions of the SDS PAGE and Western blot, because, as described below, this mAb is broadly reactive based on its ability to activate complement deposition on different strains.

Binding of anti-GNA1870 mAbs and activation of human complement deposition on the surface of live N. meningitidis *cells*

Although lack of mAb bactericidal activity did not correlate with decreased strain expression of GNA1870 as measured by Western blot, the lack of bactericidal activity could have resulted from decreased surface accessibility of the respective epitopes. Fig. 4*A* shows binding of IgG Abs to the cell surface of live meningococci as measured by flow cytometric detection of indirect immunofluorescence. Data are shown for strain MC58, the mutant MC58 knockout, MC58ΔGNA1870, and a representative strain expressing a subvariant of variant 1 GNA1870 (strain 4243). The positive control anti-group B polysaccharide mAb bound to both the parent and mutant MC58 strains (*row 1*). The positive control for the capsular group C strain 4243, a mAb-specific PorA (P1.2), also showed good binding. *Row 2* shows the respective binding of a 1/10 dilution of a negative control antiserum from mice immunized with adjuvant alone (filled area) or the corresponding binding of a 1/250 dilution of polyclonal antiserum prepared from mice immunized with variant 1 rGNA1870 (open area). With the anti-rGNA1870 antiserum, there was significant increased binding to MC58, and to a lesser extent with strain 4243, as demonstrated by a shift to the right, indicating higher fluorescence intensity. This shift was not detected with the GNA1870 knockout. *Rows 3–6* show the respective binding of JAR1, 3, 4, and 5, each tested at 50 and 0.4 μg/ml. With strain MC58, all four mAbs showed modest binding. However, with strain 4243, the mAbs showed no significant binding above background, with the possible exception of JAR5. Note also that the respective binding of three of the mAbs to MC58 was similar when tested at concentrations of 50 or 0.4 μg/ml (125-fold lower). These results suggest that even at the lowest concentration tested, JAR1, 3, and 5 are present in excess of the respective surface-accessible GNA1870 epitopes in MC58. In contrast, with JAR4 there was less binding at the lower mAb concentration than at the higher concentration. This result suggests that JAR4 may have lower avidity for binding to GNA1870 than that of the other mAbs, or that the epitope recognized by JAR4 in strain MC58 is relatively less accessible.

The ability of the mAbs to activate human C3b deposition on the bacterial surface of strains MC58, MC58ΔGNA1870, and 4243 is shown in Fig. 4*B*. There was no evidence of complement deposition when the bacterial cells were incubated with complement together with a 1/100 dilution of the negative control antiserum (*row 2*). The addition of complement to a 1/250 dilution of the polyclonal anti-GNA1870 antiserum (*row 2*) elicited strong deposition of C3b on the surface of strains MC58 and 4243, as shown by an increase in the percentages of bacteria showing strong immunofluorescence with the anti-C3c Ab, which recognizes C3b and iC3b. With strain MC58, as little as 0.5 μg/ml JAR1, JAR3, or JAR4 (*rows 3*, *4*, and *5*, respectively) activated C3b deposition. In contrast, JAR5 elicited minimal activation of complement deposition, even when tested at mAb concentration of 50 μg/ml (Fig. 4*B*, *row 6*). With strain 4243, there was no significant complement deposition elicited by JAR1 or JAR5 (Fig. 4*B*, *rows 3* and *6*, respectively). However, as little as 0.5 μg/ml JAR3, or 50 μg/ml JAR4, elicited strong complement deposition on strain 4243 (*rows 4* and *5*, respectively) despite minimal binding of these mAbs to this strain.

Table IV summarizes the data on mAb binding to the cell surface and activation of complement deposition of all nine strains. A number of the mAbs were able to activate human C3b deposition on strains that were resistant to bacteriolysis mediated by human complement. For example, JAR4, which was poorly bactericidal (Table II), and showed minimal surface binding to heterologous strains expressing subvariants of GNA1870 variant 1 protein, activated human C3b deposition on all nine strains. JAR5, which showed similar or better surface binding as that of JAR4, elicited minimal complement deposition with any of the strains tested other than M4105.

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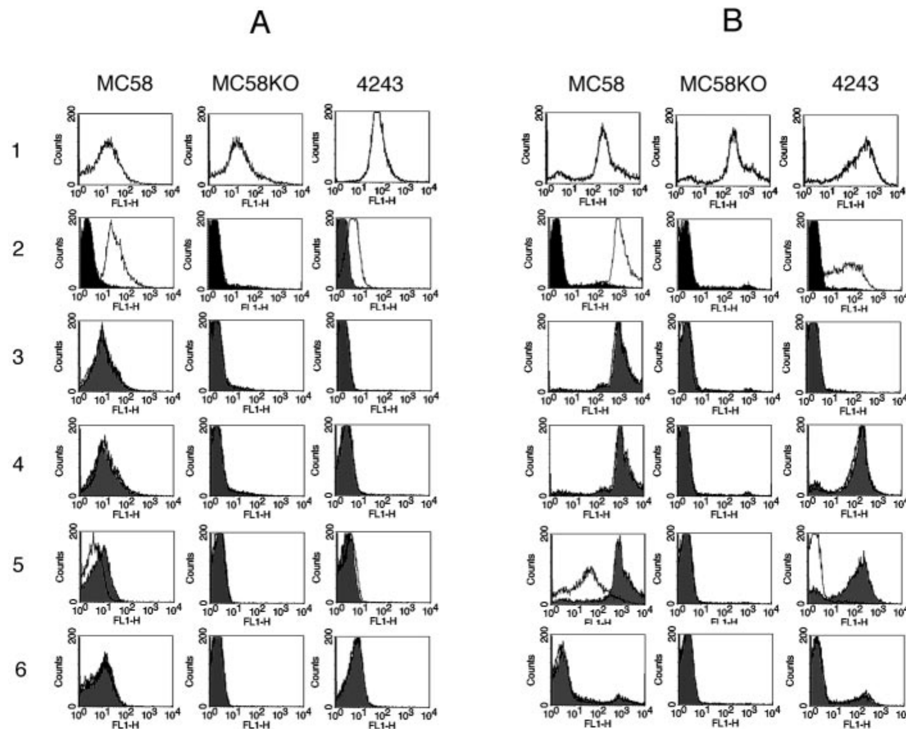

FIGURE 4. *A*, Binding of anti-GNA1870 Abs to live encapsulated *N. meningitidis* strains as measured by indirect fluorescence flow cytometry. Strain MC58 (ET5 complex). Strain MC58ΔGNA1870. Strain 4243. Positive control mAbs (*row 1*): anti-group B polysaccharide (MC58 and MC58KO) and anti-PorA (P1.2) (4243). Test antisera or mAbs: 1/10 dilution of negative control antiserum from mice immunized with adjuvant alone (filled area) and 1/250 dilution of anti-rGNA1870 polyclonal antiserum (open area) (*row 2*). JAR1 (*row 3*), JAR3 (*row 4*), JAR4 (*row 5*), and JAR5 (*row 6*). The mAbs were each tested at concentrations of 50 μg/ml (filled area) and 0.4 μg/ml (open area). *B*, Activation of human C3b complement deposition on the surface of live encapsulated *N. meningitidis* cells as determined by indirect fluorescence flow cytometry. Strain MC58. Strain MC58ΔGNA1870. Strain 4243. Positive control mAbs (*row 1*) are as described in *A*. Test antisera or mAbs: complement plus either 1/100 dilution of negative control serum (filled area) or 1/250 dilution of anti-rGNA1870 polyclonal antiserum (open area) (*row 2*). Complement plus JAR1 (*row 3*), JAR3 (*row 4*), JAR4 (*row 5*), or JAR5 (*row 6*). The mAbs were tested at concentrations of 50 μg/ml (filled area) and 0.5 μg/ml (open area).

Passive protection in the infant rat meningococcal bacteremia model

Abs that activate complement-mediated bactericidal activity against a strain can be presumed to confer protective activity (25). However, the converse is not true, in that some Abs that are not bactericidal can confer protection. Table V summarizes the results of passive experiments in infant rats treated with JAR1 or JAR3 and challenged with group B strain NZ98/254 (Expt. 1), or group C strain 4243 (Expt. 2). Both strains were resistant to bacteriolysis by these two anti-GNA1870 mAbs (Table II). JAR1 conferred

Table IV. *Ab binding and human complement deposition by anti-GNA1870 mAbs as measured by flow cytometry*[a]

		Mouse Polyclonal Anti-GNA 1870		mAb JAR1 IgG3		mAb JAR3 IgG3		mAb JAR4 IgG2a		mAb JAR5 IgG2b	
Strain	ET Cluster	Ab Binding	C3b Deposition	Ab Binding	C3b Deposition	Ab Binding	C3b Deposition	Ab Binding	C3b Deposition	Ab Binding	C3b Deposition
MC58[b]	ET5 complex	3^+	3^+	3^+	3^+	3^+	3^+	1^+	2^+	3^+	–[d]
MC58 GNA 1870KO	ET5 complex	–	–	–	–	–	–	–	–	–	–
H44/76	ET5 complex	3^+	3^+	3^+	3^+	3^+	3^+	3^+	2^+	3^+	–
Cu385	ET5 complex	3^+	3^+	3^+	3^+	3^+	3^+	3^+	2^+	3^+	–
BZ83	ET5 complex	3^+	3^+	3^+	ND	3^+	ND	2^+	2^+	3^+	–
4243	ET37 complex	3^-	3^+	–	–	–	3^+	–	2^+	3^-	–[d]
M6190	ET37 complex	3^-	2^+	–	–	–	–	–	1^+	–	–
NZ98/254	Lineage 3	3^+	2^+	–	1^+	3^+	3^+	–	2^+	3^+	–
M1390	Lineage 3	3^+	2^+	–	1^+	3^-[c]	3^+	2^-[c]	2^+	3^+	–
M4105	Lineage 3	3^+	3^+	3^-[c]	3^+	3^-[c]	3^+	1^-[c]	2^+	3^+	3^+

[a] Binding was performed at mAb concentrations of 50, 10, and 0.4 μg/ml, and at polyclonal dilutions of 1/10, 1/250, and 1/1250. C3b deposition was performed at mAb concentrations of 50, 5, and 0.5 μg/ml, and at polyclonal dilutions of 1/100 and 1/1000. Both IgG binding and C3b deposition are scored as follows: −, no significant increase in fluorescence above that of negative control; 1^+, ≥10-fold increase in intensity of fluorescence only at the highest Ab concentrations tested; 2^+, ≥10-fold increase in intensity of fluorescence at both the highest and intermediate Ab concentrations tested; 3^+, ≥10-fold increase in intensity of fluorescence at all concentrations tested.

[b] Source for DNA sequence used to prepare rGNA1870 that was used as immunogen to prepare mAbs (see *Materials and Methods*).

[c] 3^-, 2^-, or 1^-, Signifies that IgG binding or C3b deposition was significantly above background, but less than a 10-fold increase at all concentrations tested, the lowest and intermediate concentrations tested, or the highest concentration tested, respectively.

[d] Although scored as negative, a small proportion of the cells showed intensity of fluorescence of 10^2 to 10^3 at all concentrations tested (e.g., see Fig. 4*B*).

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Table V. *Passive protection of infant rats challenged with* N. meningitides[a]

Experiment (Challenge Strain)	Antiserum or mAb	Dose of mAb (μg/rat) or Serum Dilution	Blood Culture at 18 h	
			No. positive/total	CFU/ml (geometric mean)
Expt. 1 (B:NZ98/254)	Negative control serum	1/10	9/10	101,300
	Polyclonal anti-rGNA1870	1/10	0/5	<1
	Anticapsular mAb	5 μg	0/5	<1
Anti-GNA1870 mAbs	JAR1	50 μg	5/5	155
	JAR1	5 μg	8/10	1,611
	JAR3	5 μg	4/10	6
Expt. 2 (C:4243)	Negative control serum	1/10	5/5	293,070
	Polyclonal anti-rGNA1870	1/10	0/5	<1
	Human polyclonal anticapsular	0.04 μg	0/5	<1
Anti-GNA1870 mAbs	JAR1	50 μg	8/8	198,205
	JAR1	5 μg	8/8	90,210
	JAR3	5 μg	2/8	2

[a] Five- to 7-day-old infant rats were treated i.p. with different doses of mAbs or antisera. Two hours later, the animals were challenged i.p. with 4.2×10^3 CFU of group B strain NZ98/254 (Expt. 1), or 1×10^3 CFU of group C strain 4243 (Expt. 2). Quantitative blood cultures were obtained 18 h later. Animals treated with JAR1 or JAR3 and challenged with NZ98/254 showed significantly lower geometric mean CFU/ml than that of negative control animals (both doses of JAR1, $p \leq 0.04$; and JAR3, $p < 0.001$). In animals challenged with group C strain 4243, JAR3 ($p < 0.001$), but not JAR1 ($p > 0.2$) gave protection against bacteremia.

protection only against strain NZ98/254, and not against strain 4243. In contrast, JAR3 passively protected rats from bacteremia caused by either strain. The respective results paralleled the ability of these two mAbs to activate C3b deposition (JAR1 with strain NZ98/254, but not 4243, and JAR3 with both strains; see Table IV and Fig. 4*B*).

In other experiments not shown with animals treated with JAR4 or JAR5, passive protective activity also correlated with the ability of the mAb to activate C3b deposition on the bacterial cell surface. For example, a dose of 50 μg/rat JAR5, which is a poor activator of complement deposition (Table IV), did not protect against bacteremia in animals challenged by strain 4243 (10 of 10 rats with bacteremia with a geometric mean CFU/ml 5,062, which was not significantly different from that of the negative control animals (10 of 10 animals with bacteremia and a geometric mean CFU/ml 4,279)). Similarly, 50 μg/rat JAR5 did not protect against challenge by strain NZ98/354 (8 of 8 rats with bacteremia with a geometric mean CFU/ml 10,235 as compared with 10 of 10 negative control rats with bacteremia and a geometric mean CFU/ml 3,744). In contrast, in the same experiments, JAR4, a strong activator of C3b deposition on both of these strains, conferred passive protection against both strains. In animals challenged with strain 4243, 0 of 10 rats given a dose of 50 μg/rat JAR4 had bacteremia, and 4 of 10 rats given a dose of 5 μg/rat had bacteremia, as compared with 10 of 10 rats treated with a negative control serum ($p \leq 0.01$). In animals challenged with strain NZ98/354, 2 of 9 rats given a dose of 50 μg/rat developed bacteremia, and 0 of 8 rats given a dose of 5 μg/rat had bacteremia, as compared with 10 of 10 treated with the negative control ($p < 0.001$).

Discussion

GNA1870 is a new meningococcal vaccine candidate that was identified by genome mining by Masignani et al. (24). The Ag was shown to be expressed by all *N. meningitidis* strains tested, and polyclonal anti-GNA1870 raised in mice were bactericidal. The authors noted two potential limitations of GNA1870 as a vaccine candidate: expression of the protein varied with some strains expressing low amounts, and there was extensive amino acid sequence diversity. However, a few strains expressing low amounts of the protein were found to be susceptible to bactericidal activity of polyclonal anti-GNA1870 Abs, and the amino acid sequence variability among strains appeared to be conserved within three variant groups.

In the present study, we assessed the vaccine potential of GNA1870 further by producing a panel of four murine anti-rGNA2132 mAbs that recognize surface-exposed GNA1870 epitopes expressed by *N. meningitidis* strains carrying subvariants of variant 1 GNA1870 proteins. All four mAbs bound to the surface of group B *N. meningitidis* strain MC58 (Fig. 3*A*), and were bactericidal against MC58 when tested with rabbit complement (Fig. 1*A*). Three of the mAbs also were bactericidal with human complement (Fig. 1*B*). The respective activities of the four mAbs against the other ET5 complex strains were similar to those observed with MC58 (Tables II and IV). However, in binding and functional assays with *N. meningitidis* strains from the other hypervirulent genetic clusters, the activities of the mAbs were quite different from each other (Tables II and IV). The most notable differences were that the surface-accessible epitopes recognized by the mAbs in these strains were less available for binding than in the ET5 strains (compare relative binding to MC58 (Fig. 4*A*) with that of the heterologous strains from cluster ET37 (Fig. 4*A*) or lineage 3 (Table IV), and the general absence of bactericidal activity with human complement against strains that were not ET5 complex (Table II)).

Despite the absence of bactericidal activity with human complement, the most important findings of this study are that even low amounts of surface binding with some of the mAbs were sufficient to activate C3b deposition on the bacterial surface (see, for example, JAR3 and JAR4 and strain 4243; Fig. 4*B*). Also, activation of C3b deposition on strains resistant to complement-mediated bacteriolysis predicted the ability of the mAb to confer passive protection against meningococcal bacteremia in the infant rat model. These results extend previous observations made by this laboratory with polyclonal Abs raised against another meningococcal lipoprotein vaccine candidate, GNA2132, which indicated that in the absence of bactericidal activity, activation of C3b deposition predicted passive protection against meningococcal bacteremia in the infant rat model (23). Although not measured directly, the most likely mechanism conferring protection in the absence of complement-mediated bacteriolysis is opsonization because both C3b and the Fc portion of IgG Ab are known ligands for interacting with receptors on phagocytic cells.

In the present study, we found that an anti-GNA1870 mAb could show similar respective binding to the surface of two encapsulated *N. meningitidis* strains and be a strong activator of C3b

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deposition on both strains, but that complement activation progressed to insertion of a functionally active membrane attack complex and killing of one strain, but not of the other strain. An example is JAR3, which bound and activated complement deposition with strains MC58 (bactericidal) and NZ98/254 (not bactericidal). Note that both of these strains are readily killed by anticapsular Abs and human complement (23). Therefore, resistance of strain NZ98/254 to anti-GNA1870 complement-mediated bacteriolysis was not a result of an intrinsic resistance of that strain to killing by human complement.

For some anti-GNA1870 mAbs, lower surface binding to a strain correlated with less C3b deposition and failure to proceed to bacteriolysis. An example is JAR4, which at a concentration of 0.5 μg/ml showed less binding to MC58 and lower C3b deposition than that of JAR1 or JAR3 (Fig. 4, *A* and *B*). These functional differences in mAb activity may reflect differences in Ab avidity, or in the density or spatial localization of the epitopes recognized by the different mAbs.

Of particular interest are our findings that a combination of two mAbs, each of which individually was not bactericidal with human complement, had potent bactericidal activity when tested in combination (Table III). Thus, failure of Ab binding to activate complement that progresses to a functionally active membrane attack complex can be compensated for by binding of two different mAbs with different isotypes and epitope specificities. The enhanced bactericidal activity observed with a combination of two mAbs against strain 4243 parallels the high bactericidal activity of the polyclonal anti-GNA1870 antiserum observed with this strain (Table II). Although the combination of mAbs also was bactericidal against strain NZ98/254, the polyclonal anti-GNA1870 antiserum prepared for this study was not bactericidal against this strain when tested with human or rabbit complement (Table III). The bactericidal activity of the combination of anti-GNA1870 mAbs with strain NZ98/354 is consistent with previous data showing high bactericidal activity against this strain with a different pool of mouse polyclonal anti-GNA1870 antiserum and a different pool of rabbit complement (22). Why the polyclonal antiserum used in the present study was not bactericidal against strain NZ98/254 is not known.

Variant 1 GNA1870 was previously reported to be the most common variant group among disease-producing *N. meningitidis* group B strains, and was carried by all *N. meningitidis* strains tested from hypervirulent lineage ET-5 complex strains, and most lineage 3 strains (24). Our data indicate that anti-GNA1870 Abs prepared to a variant 1 GNA1870 recombinant protein are broadly cross-reactive with encapsulated *N. meningitidis* strains expressing subvariants of GNA1870 variant 1 protein despite the presence of amino acid polymorphisms and/or low surface accessibility of certain GNA1870 epitopes. Furthermore, the Abs conferred broad protection against *N. meningitidis* variant 1 strains resistant to complement-mediated bacteriolysis. These data provide further support for GNA1870 as a highly promising vaccine candidate for prevention of group B meningococcal disease caused by GNA1870 variant 1 strains.

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