Protective Immunization against Group B Meningococci Using Anti-Idiotype Mimics of the Capsular Polysaccharide

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Use of the serogroup B meningococcal capsular polysaccharide (MenB CP) as a vaccine is hampered by the presence of epitopes that cross-react with human polysaccharide. As non-cross-reactive, protective capsular epitopes have also been described, we set out to develop protein mimics of one of such epitopes using as a template a highly protective mAb (mAb Seam 3) raised against a chemically modified form of the MenB CP (N-Pr MenB CP). Using phage display, anti-idiotype single-chain Ab fragments (scFvs) were obtained from spleen cells of mice immunized with the Seam 3 mAb. Two Seam 3-specific scFvs competed with N-Pr MenB CP for binding to either mAb Seam 3 or rabbit Abs present in typing sera. Moreover, in mice and rabbits the scFvs elicited the production of Abs reacting with both N-Pr MenB CP and whole meningococci, but not with human polysaccharide acid. These scFv-induced Ab responses were boostable and of the Th1 type, as shown by a predominance of IgG2a. In addition, passive immunization with sera from scFv-immunized animals partially protected neonatal mice from experimental infection with group B meningococci. In conclusion, we have produced anti-idiotype scFvs that mimic a protective MenB CP epitope and may be useful in the development of an alternative group B meningococcal vaccine. The Journal of Immunology, 2004, 172: 2461–2468.

Neisseria meningitidis is one of the most common causes of bacterial meningitis and septic shock in infants and young adults. Mortality rates from meningococcal meningitis range from 5–10%, and neurological sequelae are present in up to 25% of the survivors (1, 2). The main virulence factor of these organisms is the capsular polysaccharide (CP),2 which protects against complement-mediated bacteriolysis and phagocytosis (3). Based on chemically and antigenically distinctive capsular properties, N. meningitidis can be divided into 12 serogroups, with serogroups A, B, C, Y, and W-135 accounting for virtually all disease-producing isolates (4). CP-protein conjugate vaccines can induce effective Ab responses and therefore hold great promise for the control of infections caused by groups A, C, W-135, and Y (5).

In contrast, group B N. meningitidis (MenB), which is responsible for up to 80% of the total cases of disease in industrialized countries, has been resistant to any attempt to develop a capsule-based vaccine due to the poor immunogenicity of the CP even after protein conjugation. This is probably due to the similarity between the MenB CP and a polymer expressed on the neuronal cell adhesion molecule of mammalian tissues (6). Both polysaccharides are α(2→8) homoliner polymers of N-acetylneuraminic acid (polysaccharide acid), although the bacterial CP is longer than human polysaccharide acid (~200 vs 50 residues, respectively). Therefore, the MenB CP is a self-Ag unable to stimulate an immune response and with the potential to induce autoimmunity if used as a vaccine.

A chemically modified MenB CP, obtained by removal of N-acetyl (N-Ac) and substitution with N-propionyl (N-Pr) groups, can elicit, after conjugation to a protein, bacterialid Abs that passively protect animals from experimental MenB infection (7). It is believed that the N-Pr CP somehow mimics antigenic determinants that are present on the bacterial surface, but are infrequently expressed on the purified N-Ac CP. The N-Pr CP, however, also elicits a small subset of autoreactive IgGs. Although the concentration and/or avidity of the latter are generally low (8), it may be difficult to prove that such autoantibodies are absolutely innocuous. By the use of mAbs raised against either the N-Pr or the N-Ac CP, at least two different classes of capsular epitopes have been defined, both of which can be found on the intact bacterial surface. One class, expressed mainly on N-Ac polysaccharide, is cross-reactive with human tissue, whereas the other, which predominates in N-Pr polysaccharide acid, is protective and non-cross-reacting (9–11).

It is well established that protein molecules, such as peptides and anti-idiotype Abs, can mimic functional features of carbohydrate Ags, including their ability to induce the production of specific Abs (reviewed in Ref. 12). The ability of murine monoclonal anti-idiotype Abs to function as surrogate vaccines against several different encapsulated pathogens has been shown in the past (13–15). More recently, it was shown that anti-idiotype single-chain Ab fragments (scFvs) are sufficient to induce protective, capsule-specific Ab responses (16). These recombinant fragments are attractive, because they can be easily manipulated for different purposes, including DNA vaccination and/or fusion with cytokines or immunogenic peptides. In the present study, using an appropriate mAb as a template, we generated scFv mimics of a protective, non-cross-reactive epitope of the MenB CP. These constructs were able to induce anti-meningococcal Ab responses in animals and therefore may be useful in the development of a safe MenB vaccine.

Materials and Methods

Bacterial strains and reagents

Three encapsulated group B N. meningitidis strains (2996, MC58, and 8047) and the unencapsulated M7 strain (17) were provided by M. Giuliani

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2 Abbreviations used in this paper; CP, capsular polysaccharide; KLH, keyhole limpet hemocyanin; MenB, serogroup B meningococcal; N-Ac, N-acetyl; N-Pr, N-propionyl; PMN, polymorphonuclear leukocyte; scFv, single-chain Ab fragment; TT, tetanus toxoid.
(Chiron, Siena, Italy). Purified N-Pr MenB, N-Pr MenB, and biotin-conjugated N-Pr MenB CPs, prepared as previously described (9, 18), were provided by A. Bartolini (Chiron). Vaccines consisting of N-AC MenB and N-Pr MenB CPs conjugated to tetanus toxoid (N-AC MenB-TT and N-Pr MenB-TT, respectively) were prepared as previously described (9). Brieﬂy, after partial acid hydrolysis of the polysaccharides, intermediate size (9–25 repeating units) oligosaccharides were separated by ion exchange chromatography, oxidized, and conjugated with TT (obtained from Calbiochem, La Jolla, CA). After puriﬁcation by gel ﬁltration, saccharide to protein ratios, determined as previously described (9), were 0.19 and 0.22 for N-Pr MenB-TT and N-AC MenB-TT, respectively. Puriﬁed murine Seam 3 mAb was provided by M. Mariani (Chiron). The Seam 3 mAb is an IgG2b raised against N-Pr MenB that strongly reacts against encapsulated MenB to produce complement-mediated bacteriolysis and has no autoantibody reactivity (9). Rabbit MenB-speciﬁc typing serum was purchased from Difco (distributed by Voigt Global Distribution, Kansas City, MO).

Preparation of Ab fragments
Preparation of Ab fragments was accomplished as previously described (16, 19), with minor modiﬁcations, using libraries of ﬁlamentous phages expressing murine scFvs on their surface. Brieﬂy, female BALB/c mice (8 wk old) were s.c. injected on days 0 and 15 with 50 μg of mAb Seam 3 in 0.2 ml of CFA (Difco) and on days 21 and 28 with the same Ag dose in 0.2 ml of IFA (Difco). A ﬁnal booster injection (50 μg of Ag in saline) was given i.p. on day 35, and 3 days later spleen cells were obtained. After incubating these cells on Seam 3-coated plates, RNA was extracted from adherent cells. After reverse transcribing puriﬁed mRNA with random hexadecamer primers, a commercial system (Recombinant Phage Ab System; Amersham Pharmacia Biotech, Milan, Italy) was used to clone and express Ab fragments. Recombinant phages displaying scFv Abs on their tips were selected after absorption of the library with an irrelevant IgG2b mAb (mouse IgG2b MOPC 141; Sigma-Aldrich, Milan, Italy) as previously described (16, 19) by three rounds of panning on cultured adherent cells. After reverse transcribing puriﬁed mRNA with random hexadecamer primers, a commercial system (Recombiant Phage Ab System; Amersham Pharmacia Biotech, Milan, Italy) was used to clone and express Ab fragments. Recombinant phages displaying scFv Abs on their tips were selected after absorption of the library with an irrelevant IgG2b mAb (mouse IgG2b MOPC 141; Sigma-Aldrich, Milan, Italy) as previously described (16, 19) by three rounds of panning on culture ﬂasks that had been previously coated with mAb Seam 3. Screening of isolated colonies was performed by phage ELISA as previously described (16, 19). Seam 3-speciﬁc scFvs and the irrelevant H6 scFv (19, 20) were produced and puriﬁed as previously described (16, 19). Purity was checked using SDS-PAGE gels staining of overloaded SDS-polyacrylamide gels as previously described (16). Under these conditions, only a single band with the expected molecular mass of 30 kDa was observed.

Restriction ﬁngerprinting
To ascertain whether different clones from the same library shared a common ancestry, restriction analysis was performed on ampliﬁed DNA fragments. To amplify a region containing the scFv genes, 25 μl of a master PCR mixture was prepared with the M13 sense primer 5¢-GGTAACGCAGGGTTTTCC-3¢ and the antisense M13 primer 5¢-GGTAACGCAGGGTTTTCC-3¢ and the antisense M13 primer 5¢-GGTAACGCAGGGTTTTCC-3¢. The PCR products were run with a 10% sucrose and 4% polyvinylalcohol gel (Seakem LE, Cambrex Bio Science). These sequences have been deposited with GenBank (accession no. AY734127 and AY734128).

Inhibition ELISA
To study the ability of anti-idiotype scFvs to compete with N-Pr MenB CP for binding to MenB-speciﬁc Abs, an inhibition ELISA was used. Brieﬂy, wells of microtiter plates (Nunc, distributed by M. Brunelli, Milan, Italy) were coated overnight at 4°C with hen’s egg avidin (ExtrAvidin; Sigma-Aldrich) at a concentration of 4 μg/ml in PBS (0.01 M phosphate and 0.15 M NaCl, pH 7.2). After washing, the wells were incubated for 2 h at 37°C with biotinylated N-Pr MenB CP (0.5 μg/ml) in PBS containing 1% BSA (PBS-BSA; Sigma-Aldrich). After washing with PBS containing 0.05% polyoxyethylene-sorbitan monolaurate, the plates were blocked with 1% gelatin in PBS for 3 h and ﬁxed with a 10% sucrose and 4% polyvinylpyrrolidone solution. Test samples, consisting of mixtures of MenB-speciﬁc Abs and putative inhibitors, were added, and the plates were incubated for 2 h at 37°C. Positive and negative controls consisted of soluble N-Pr MenB and the group-speciﬁc polysaccharide of group B streptococci, obtained as previously described (21). After washing, bound Abs were detected by HRP-conjugated anti-mouse or anti-rabbit IgG Abs (Amersham Pharmacia Biotech), followed by 2% (w/v) 2,2¢-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) in 0.05 M citric acid. Reading was performed at 405 nm on a microplate reader. Percent inhibition was calculated by comparing the absorbance values of wells with and without the inhibitors.

Immunization with scFvs
Female BALB/c mice (6 wk old) or New Zealand rabbits were used for immunization with scFvs. In the case of mice, a ﬁrst s.c. injection of 50 μg in 0.2 ml of CFA was followed, on days 21 and 42, by two additional s.c. injections of the same Ag dose in 0.2 ml of IFA. Rabbits underwent a similar immunization regimen, except that they received 500 μg of Ag for each s.c. injection at multiple sites. Sera were obtained from the retro-orbital plexus (mice) and the saphenous vein (rabbits) at the indicated times and were tested as described below.

Control mice were immunized three times s.c. with N-Pr MenB-TT or N-AC MenB-TT (4 μg of saccharide/ScFv) in CFA (day 0) or IFA (days 21 and 42). In additional experiments mice were immunized with the G1 scFv conjugated with keyhole limpet hemocyanin (KLH; Pierce, distributed by Celbio, Milan, Italy). The scFv (5 mg) was mixed with KLH (20 mg) in phosphate buffer (0.1 M, pH 6.8) containing 0.5% glutaraldehyde (Sigma-Aldrich). The reaction was stopped after 30 min by the addition of 1 M glycine (Sigma-Aldrich), followed by extensive dialysis using 50,000 kDa cutoff membranes. The scFv-KLH conjugate was adsorbed to Al(OH)3 gel (Sigma-Aldrich). A group of ﬁve mice each received scFv-KLH on days 0 and 21 with 0.1 ml of the alum-adsorbed conjugate corresponding to 50 μg of the G1 scFv. Another group of ﬁve mice received 50 μg of the G1 scFv in CFA on day 0 and the same Ag dose in IFA on day 21.

N-Pr MenB CP and whole bacteria ELISA
Abs in sera from scFv-immunized animals were assayed for binding to N-Pr MenB CP or to whole bacteria using ELISA. Microtiter plates were coated with avidin, followed by biotinylated N-Pr MenB, as described above (see Inhibition ELISA). To sensitize plates with whole bacteria, strains 2996, MC58, 8047, and M7 were grown at 37°C in 5% CO2 in Muller-Hinton broth to the early exponential phase, washed in PBS, heat-inactivated (56°C for 30 min), and lyophilized. Five micrograms of lyophilized bacteria per well were used to sensitize the plates. After the addition of diluted mouse or rabbit sera, bound Abs were detected with HRP-conjugated polyclonal anti-mouse or anti-rabbit IgG (Amersham Pharmacia Biotech). Isotype-speciﬁc HRP-conjugated Abs (Vector Laboratories, distributed by Labtek, Milan, Italy) were used to detect mouse IgM, IgG1, IgG2a, IgG2b, and IgG3.

Bactericidal and opsonophagocytosis assays
Bactericidal activity was assayed in serum samples from scFv-immunized animals using 96-well microtiter plates, with modiﬁcations of a previously described procedure (22). Groups of rabbits (New Zealand white rabbits) were inoculated s.c. on day 21 with 25 μg of the G1 scFv, and test organisms were grown overnight on chocolate agar plates (Oxoid, Milan, Italy). Approximately ﬁve colonies were inoculated into Muller-Hinton broth, and test organisms were grown to an A560 of 0.24 (early log phase). After washing, 1000 CFU in 25 μl of PBS-BSA were added to 25 μl of undiluted, freshly thawed baby rabbit complement (Cedarlane, distributed by Celbio) or heat-inactivated complement and to 25 μl of diluted test sera. After a 1-h incubation with shaking, an aliquot (10 μl) of the reaction mixture from each well was transferred onto chocolate agar plates, and CFU were counted after an overnight incubation at 37°C in 5% CO2 and 95% humidity. Each assay was conducted in triplicate. Bactericidal activity was expressed as the percent variation in CFU numbers observed in the presence of fresh complement relative to heat-inactivated complement.

Opsonophagocytic activity was measured in serum samples in an identical way, except that 5 ¥ 105 human polymorphonuclear leukocytes (PMNs) were added to the mixtures containing serum dilutions, bacteria, and complement. To isolate PMNs, the peripheral blood from adult healthy donors was centrifuged on a Polymorphoprep gradient (Sentinel CH, Milan, Italy), as described by the manufacturer.

Detection of cross-reactive Abs
Ab binding to mammalian polysaccharide was assayed in sera from immunized mice using the human neuroblastoma CHF 212 cell line (provided by A. Pession, Università di Bologna, Bologna, Italy), which expresses high levels of polysaccharide (23). Cells from nearly confluent cultures were collected, washed in PBS, and counted. One aliquot was incubated with exoenzymeunimidine (Sigma-Aldrich; 150 U/ml) cells/mL) at 30°C for 2 h to cleave surface polysaccharide. The cells from each aliquot were then
dispensed into the wells of microtiter plates (10^5 cells/well) and fixed for 1 h at 20°C with 2% paraformaldehyde (Sigma-Aldrich) in PBS. After blocking with 1% gelatin (1 h at 37°C) and washing with PBS-BSA, diluted serum samples were added to the wells, and the ELISA was developed as described above. The Seam 26 mAb (9), known to react against mammalian polysialic acid, was used as a positive control.

Passive immunization with sera

To study the protective effects of passively administered sera from immunized animals, a neonatal mouse model of systemic meningococcal infection was used. Neonatal (<48-h-old) BALB/c mice were randomly assigned to control or experimental groups (30/group), marked, and kept with the mother. Pups were inoculated s.c. with 30^6 CFU of strain 2996 corresponding, respectively, to 50 and 90% lethal doses. Mortality was assessed every 24 h for 5 days. Deaths rarely occurred after the 48-h challenge. N-Pr MenB was used as a positive control. Negative controls consisted of the group-specific polysaccharide from group B streptococci or the irrelevant H6 scFv. After washing, the ELISA was developed as outlined in Materials and Methods. Percent inhibition was calculated relative to absorbance values observed in the absence of inhibitors.

Results

Production of anti-idiotypic scFvs

To produce anti-idiotypic scFvs, BALB/c mice were immunized with Seam 3, an mAb raised against N-Pr MenB CP (9). ScFv libraries displayed on filamentous phages were prepared from spleen cells of immunized mice, and individual clones were selected using Seam 3-coated plates. A total of 48 clones were obtained and two of these clones, designated G1 and B5, were selected on the basis of strong ELISA signals. As these clones were obtained from the same library, they could share clonal ancestry. To exclude this possibility, fingerprint analysis was performed on PCR-amplified DNA fragments after BstXI digestion. The restriction pattern of the G1 fragment markedly differed from that of B5 (not shown), thus excluding shared ancestry. Nucleotide sequence analysis further confirmed that G1 and B5 were different scFvs.

Inhibition ELISA

Next, it was determined whether the G1 and B5 anti-idiotypic scFvs could mimic some of the antigenic properties of the nominal Ag, i.e., the N-Pr MenB CP. First, we looked at the ability of the scFvs to compete with the N-Pr MenB CP for binding to Seam 3. Fig. 1 shows the results obtained with an inhibition ELISA using N-Pr MenB CP as the coating Ag. As expected, soluble N-Pr MenB CP, used as a positive control, inhibited Seam 3 binding, with a 50% inhibitory concentration of 10 ng/ml (Fig. 1, left panel). In contrast, the negative control, consisting of group-specific polysaccharide from group B streptococci, did not produce any inhibition at doses up to 50 μg/ml. Both the G1 and B5 scFvs could block Seam 3 binding in a dose-dependent fashion, with 50% inhibitory concentrations of 7 and 9 μg/ml, respectively. In contrast, the irrelevant H6 scFv failed to inhibit Seam 3 binding at concentrations as high as 50 μg/ml.

FIGURE 1. ScFv-mediated inhibition of Ab binding to N-Pr MenB CP-coated plates. The left and right panels show inhibition of binding of, respectively, Seam 3 mAb and MenB-specific polyclonal rabbit Abs. Mixtures of Seam 3 (250 ng/ml, final concentration) or rabbit typing serum (final dilution of 1/40) and inhibitors were incubated for 20 min at 37°C and added to N-Pr MenB CP-coated plates. Soluble N-Pr MenB CP was used as a positive control. Negative controls consisted of the group-specific polysaccharide from group B streptococci or the irrelevant H6 scFv. After washing, the ELISA was developed as outlined in Materials and Methods. Percent inhibition was calculated relative to absorbance values observed in the absence of inhibitors.

FIGURE 2. Anti-N-Pr MenB CP Ab levels in sera from scFv-immunized mice. Groups of five animals were immunized on days 0, 21, and 42 (arrows) with the anti-Seam 3 G1 or B5 scFvs or with the irrelevant H6 scFv. For comparison, two additional groups of five mice each were immunized with N-Pr MenB-TT or N-Ac MenB-TT conjugates. Sera were collected on days 0, 15, 36, and 57; diluted 1/200 (left panels) or 1/100 (right panels); and tested for binding to N-Pr MenB CP-coated plates by ELISA. This was performed as described in Materials and Methods using polyvalent, γ-chain- and μ-chain-specific anti-mouse Ig (left panels) or anti-mouse IgG1, IgG2a, IgG2b, or IgG3 (right panels) HRP-conjugated Abs. Data represent the mean ± SD of five determinations, each conducted in duplicate on a different animal. * Significantly different from anti-H6 scFv sera; §, significantly different from anti-G1 or anti-B5 scFv serum.
True Ag mimics should be capable of reacting with Ag-specific Abs raised in different animal species, because in the latter, idio
types unrelated to Ag binding are unlikely to be present. Therefore,
in additional experiments we assessed the ability of G1 and B5 to
inhibit binding of rabbit polyclonal Abs present in MenB-specific
typing sera. Fig. 1 (right panel) shows that the positive control,
consisting of soluble N-Pr MenB CP, produced up to 70% inhibi
tion of Ab binding. The G1 and B5 scFvs inhibited binding of
rabbit Abs to N-Pr MenB-coated wells by 45 and 37%, respec
tively (Fig. 1, right panel). The inability of the G1 and B5 scFvs
to completely block binding of rabbit Abs was not surprising, be
cause polyclonal Abs are expected to recognize a number of dif
erent MenB CP epitopes, whereas G1 and B5 can each mimic
only a single epitope. These data indicated that the G1 and B5
cFvs could interact with the Ab-combining sites of anti-MenB CP
Abs raised in different species and therefore mimicked the Ab-
binding properties of a relevant MenB CP epitope.

Induction of Ab responses
In additional experiments we investigated whether immunization
with the G1 and B5 scFvs could induce the production of anti-N-Pr
MenB CP Abs. Sera were collected from BALB/c mice at different
times after immunization and tested for binding to N-Pr MenB
CP-sensitized plates. Low Ab levels were detected in preimmune
sera (Fig. 2, left panels). Immunization with both the G1 and B5
cFvs induced significant elevations of Abs reacting against the
N-Pr MenB CP. In contrast, immunization with the irrelevant H6
scFv produced no variation in Ab titers. Both the G1- and B5-
induced Ab responses were boostable and predominantly of the
IgG type (Fig. 2). Isotype analysis indicated that IgG2a and, to a
lesser extent, IgG1 were the predominant Abs produced (Fig. 2,
right panels).

Next, the Ab levels measured after scFv immunization were
compared with those obtained after immunization with N-Ac
MenB or N-Pr MenB conjugate vaccines. The N-Pr MenB-TT, but
not the N-Ac MenB-TT, conjugate vaccine produced significant
elevations in anti-Pr Men B CP Ab responses that were predomi
antly of the IgG type (Fig. 2). The N-Pr MenB-TT IgG eleva
tions were higher than those induced by the G1 or B5 anti
idiotypic scFvs ($p < 0.05$; Fig. 2, left panels).

In additional experiments it was of interest to ascertain whether
the scFvs were also able to induce significant anti-N-Pr MenB CP
responses when using alum as an adjuvant in place of CFA. To this
end, multiwell plates were sensitized with different strains of
heat-killed N. meningitidis, and selected pre- and postimmune
samples from the experiments shown in Fig. 2 were tested by
ELISA. This was performed as described in Materials and Methods,
using polyvalent anti-rabbit IgG HRP-conjugated Abs. Data represent the mean ± SD of three
determinations, each conducted in duplicate on a different animal. *, Signi
fically different from anti-H6 scFv sera by ANOVA and Student-New
man-Keuls test.

In additional experiments, it was of interest to determine
whether the anti-NPr MenB CP Abs present in sera from G1-im
munized animals could bind to the surface of whole bacteria. To
this end, multiwell plates were sensitized with different strains of
heat-killed N. meningitidis, and selected pre- and postimmune
samples from the experiments shown in Fig. 2 were tested by
ELISA. The unencapsulated strain M7 was used to control for specificity of Ab binding to the bacterial capsule. As shown in Fig.

**FIGURE 2.** Binding of sera from scFv-immunized rabbits to N-Pr
MenB CP. Groups of three animals were immunized on days 0, 21, and 42
(arrows) with the anti-ScFv G1 or B5 scFvs or with the irrelevant H6
scFv. Sera were collected on days 0, 15, 36, and 57; diluted 1/200; and
tested for binding to N-Pr MenB CP-coated plates by ELISA. This was
performed as described in Materials and Methods, using polyvalent anti
rabbit IgG HRP-conjugated Abs. Data represent the mean ± SD of three
determinations, each conducted in duplicate on a different animal. *, Signi
fically different from anti-H6 scFv sera by ANOVA and Student-New
man-Keuls test.

**FIGURE 3.** Binding of sera from scFv-immunized rabbits to N-Pr
MenB CP. Groups of three animals were immunized on days 0, 21, and 42
(arrows) with the anti-ScFv G1 or B5 scFvs or with the irrelevant H6
scFv. Sera were collected on days 0, 15, 36, and 57; diluted 1/200; and
tested for binding to N-Pr MenB CP-coated plates by ELISA. This was
performed as described in Materials and Methods, using polyvalent anti
rabbit IgG HRP-conjugated Abs. Data represent the mean ± SD of three
determinations, each conducted in duplicate on a different animal. *, Signi
fically different from anti-H6 scFv sera by ANOVA and Student-New
man-Keuls test.

**FIGURE 4.** Binding of sera from scFv-immunized animals to whole
bacterial cells using different MenB strains. Serum samples from mice
(upper panel) or rabbits (lower panel) immunized with the G1 scFv were
collected on day 0 (pre-immune) or 57 (postimmune) after the beginning of
immunization. Samples from each group were diluted 1/400 and tested for
binding to bacteria-coated plates as outlined in Materials and Methods,
using polyclonal anti-mouse or anti-rabbit IgG HRP-conjugated sera. Data
represent the mean ± SD of three determinations, each conducted in du
plicate on a different serum sample. *, Significantly different from preim
mune sera by ANOVA and Student-Newman-Keuls test.
4, there was a low level of background binding to both encapsulated and nonencapsulated N. meningitidis. However, in each of the animals tested, an increased IgG binding to encapsulated bacteria was observed after immunization with the G1 anti-idiotypic scFv. No significant elevations in anti-bacterial Ab levels were detected using the unencapsulated strain. Collectively, these data indicated that the Abs induced by scFv immunization can recognize epitopes present on both the intact group B meningococcal capsule and purified N-Pr MenB CP.

Abs directed against the MenB CP are known to be highly heterogeneous and to contain both human cross-reactive and non-cross-reactive Abs. To ascertain whether the Abs induced by the G1 scFv can recognize epitopes present on both the intact group B meningococcal capsule and purified N-Pr MenB CP.

ScFv-induced priming

The ability of priming with anti-idiotypes to modify subsequent responses to the mimicked Ags has been clearly demonstrated (12, 13). It was therefore of interest to determine whether the G1 scFv was capable of priming animals for increased anti-N-Pr MenB Ab responses upon encounter with the pathogen. To this end, animals were immunized twice with different scFvs before inoculation with whole killed group B meningococci in IFA. In animals primed with the irrelevant H6 scFv, inoculation with meningococci induced low grade elevations in anti-MenB CP IgM (Fig. 6, lower panel). In contrast, brisk elevations of anti-MenB CP Abs were observed in G1-primed animals (Fig. 6). These data indicated that

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**FIGURE 5.** Cross-reactivity with polysialic acid of Abs in sera from scFv-immunized animals. Neuraminidase-treated or untreated cells from the CHP 212 human neuroblastoma cell line were fixed in the wells of microtiter plates. Serum samples from animals immunized with the G1 scFv or the N-Pr MenB-TT conjugate were collected on days 0 (preimmune) or 57 (postimmune) after the beginning of immunization. Serum samples from each group were diluted 1/20 and tested for binding to cell-coated plates by ELISA. This was performed as described in Materials and Methods using polyclonal anti-mouse or anti-rabbit IgG HRP-conjugated Abs. The positive control consisted of undiluted hybridoma culture supernatants containing the polysialic acid-specific murine mAb Seam 26. Data represent the mean ± SD of three determinations, each conducted in duplicate on a different serum animal. *, Significantly different from neuraminidase-treated cells by ANOVA and Student-Newman-Keuls test.

**FIGURE 6.** Effects of scFv-priming on Ab responses to killed whole meningococci. Groups of five mice were inoculated with the anti-Seam 3 G1 or the irrelevant H6 scFvs on days 0 and 21 and boosted with heat-killed MenB strain 2996 (1 × 10⁶ CFU) on day 42. A control group was immunized with the G1 scFv, but was not boosted with bacteria. Sera were collected on days 0, 15, 36, and 57; diluted 1/200; and tested for binding to N-Pr MenB CP-coated plates by ELISA. This was performed as described in Materials and Methods using HRP-conjugated anti-mouse Ig. Data represent the mean ± SD of five determinations, each conducted in duplicate on a different animal. *, Significantly different from anti-H6 scFv-primed sera; §, significantly different from sera of G1 scFv-primed animals that were not boosted with meningococci.
the G1 scFv mimic of the MenB CP could effectively prime mice for increased Ab responses after exposure to whole bacteria.

**Passive immunoprotection**

In additional experiments we looked at the ability of sera from scFv-immunized mice and rabbits to passively protect infant mice from lethality produced by group B meningococci. In these experiments we used a recently developed neonatal mouse model. Preliminary experiments showed that this model correlated well with the infant rat model in testing the protective activity of murine and human sera. Fig. 7 shows that sera from G1-scFv immunized animals moderately, but significantly, increased survival in pups inoculated with a 90 or 50% lethal dose of group B meningococci. These protective effects, however, were lower \((p < 0.05)\) than those produced by passive immunization with sera from N-Pr Men B-TT-immunized animals (Fig. 7, upper panels).

**Bactericidal and opsonizing activities**

The ability of sera from scFv-immunized animals to kill *N. meningitidis* was examined in vitro using complement-dependent bactericidal or opsonophagocytosis assays. In these experiments we used selected serum samples obtained during the experiments shown in Fig. 2. Serum bactericidal activity (Fig. 8, □) was expressed as the percent decrease in CFU observed in the presence of fresh complement, relative to heat-inactivated complement. Although sera obtained from N-Pr MenB-TT immunized mice showed significant bactericidal activity, no such activity was observed after immunization of mice or rabbits with the G1 scFv. However, significant increases in opsonizing activity (Fig. 8, ■) were detected in serum samples from G1-immunized animals compared with their respective preimmune controls.

**Discussion**

After the first demonstration that the antigenic functions of carbohydrates can be mimicked by anti-idiotypic Abs (24), murine mAbs have been successfully used as experimental anti-idiotypic vaccines to induce protective immunity against different encapsulated bacteria (13–15, 25). More recently, short peptides also proved capable of mimicking carbohydrate Ags (12, 26, 27). The introduction of effective, protein-conjugated, polysaccharide vaccines has alleviated the need for alternative strategies to prevent infections by many encapsulated pathogens. In the case of group B meningococcal infections, however, no effective capsule-based vaccine is available due to cross-reaction of the capsular polysaccharide with host polysialic acid.

In the present study we have developed scFv mimics of a protective capsular MenB epitope that are devoid of cross-reactivity with host polysialic acid. These anti-idiotypic mimics not only compete with the nominal Ag (i.e., N-Pr MenB CP) for binding to specific mouse or rabbit Abs, but also induce, after immunization, N-Pr MenB CP-specific serum Abs. These scFv-induced Abs had the following characteristics: 1) lack of binding to human polysialic acid; 2) binding to a capsular epitope expressed on both the intact bacterial surface and the purified capsular polysaccharide; 3)
opsonizing activity, resulting in increased bacterial killing by hu
mam PMN leukocytes; and 4) protective activity in a neonatal model of MenB disease. Moe et al. (12) and Shim et al. (10) used a strategy similar to the one described in this study to select pep

tide mimics of the MenB CP using MenB CP-specific Abs. How

ever, the immunogenic properties of these peptide mimotopes were not described. It should be noted that none of these peptides has any sequence homology with the hypervariable regions of the anti

idiotype scFvs described in this study.

Collectively, our data indicate that the anti-idiotype strategy we used was effective in mimicking the immunogenic properties of a protective capsular epitope while avoiding the risks inherent to vaccination with the whole capsular Ag, which is known to contain host cross-reactive epitopes. In fact, both whole MenB cells and the purified capsular polysaccharide express human cross-reactive epitopes. Although N-Pr derivatization of the Ag can markedly increase immunogenicity after protein conjugation (7) (Fig. 2), cross-reactive epitopes are still present on N-Pr MenB CP, as shown in immunized mice by low, but detectable, levels of anti

polysaccharide serum IgGs (Fig. 5). These data are in agreement with previously described work (8, 9). A high frequency of poly

saccharide-specific mAbs was observed in N-Pr MenB CP-immu

nized mice after fusion of spleen cells (9). Moreover, a pool of sera from N-Pr MenB TT-immunized mice showed weak, but specific, IgG binding to polysaccharide-acid-rich neural cell adhesion molecule in embryonic brain extracts and on the surface of live cells derived from a mouse pituitary tumor (8). No binding to polysaccharide was detected, however, by other tests. Collectively, published work and the present study indicate that weak, but discernible, elevations of anti-mammalian polysialic acid Abs can be induced by N-Pr MenB TT immunization. Although the neuropathological potential of these Abs has not been shown, it may be difficult to exclude that after crossing the placenta, they can somehow damage the nervous system of the developing fetus.

In the present study the scFv-induced responses were boostable and predominantly of the IgG2a type, indicating that these anti

idiotypes were capable of producing a Th1-like response. This feature is of interest, because capsular polysaccharides are incap

able of inducing Th1-dominant responses even after protein con

jugation. In fact, although protein conjugation does confer T-de

pendent help, conjugate vaccines have limited ability to induce the Th1-associated murine IgG2a isotype, while inducing predomi

nantly IgG1 and IgG3 isotypes (28). A Th1 response with the expression of IgG2a would be desirable because IgG2a and IgG3 isotypes have been reported to be particularly effective in confer

ring protection against encapsulated organisms (29). In addition, the scFv immunization primed the mice for a MenB-specific re

sponse that was rapidly activated upon encounter with whole MenB. These data suggest that scFv immunization generates a memory B cell population that can be expanded by encounter with MenB bacteria.

However, some limitations of the anti-Id strategy used in this study need to be pointed out. First, the protective activities of the sera from scFv-immunized animals were only moderate in degree and considerably lower that those produced by the vaccine in mAb, used as a template to develop the scFv mimics. Second, the scFv-

induced sera had no significant bactericidal activity, whereas Seam 3 is highly bactericidal. The reasons underlying these phenomena are not clear, but are probably related to insufficient concentration and/or affinity of the Abs induced by the scFv immunization. Dif

ferent approaches are being used to overcome these problems, all taking advantage of the ease with which the Ab fragments used can be manipulated by molecular techniques. Higher Ab responses could conceivably be obtained by expressing the scFvs as fusion proteins containing T cell epitopes or adjuvants, such as IL-12 or other cytokines. Indeed, IL-12 treatment was shown to dramati

cally enhance Ab responses and, in particular, IgG2a responses to both unconjugated and protein-conjugated capsular polysaccharide vaccines (28). New DNA immunization strategies are also likely to result in enhanced anti-scFv responses due to increased in vivo Ag persistence and the inclusion of DNA immunostimulatory se

quences in the plasmid used for vaccination. It was recently shown that DNA vaccination with mimotope-encoded plasmid induced strong IgG2a anti-carbohydrate responses (30).

An additional strategy to increase the size and affinity of Ab responses is the development of further refined, second-generation mimics that would more closely resemble, from a structural stand

point, the natural Ag. Recent advances in structural chemistry in

volving x-ray and nuclear magnetic resonance spectroscopy may soon allow the creation of more “faithful” molecular mimics by design (31). Finally, the scFv described in this study may be used as fusion proteins in conjunction with other meningococcal Ags. Recently, promising vaccine candidates have been identified using whole genome sequencing of group B N. meningitidis (32). As all group B strains are sensitive to anti-capsular Abs, the inclusion of a capsule-based immunogen in such vaccines may help prevent bacterial escape due to the emergence of mutant strains in vacci

nated populations.

References


tococcal infection by maternal vaccination with recombinant anti-idiotypes. Nat. Med. 4:705.


