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Molecular analysis of monoclonal antibodies to group variant capsular polysaccharide of *Neisseria meningitidis*: recurrent heavy chains and alternative light chain partners

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Abstract

We determined the molecular sequence of monoclonal antibodies (mAbs) to serogroups B and C capsular polysaccharides (PS) of *Neisseria meningitidis*. *N. meningitidis* infections are a leading cause of bacterial septicemia and meningitis in humans. Antibodies to PS are fundamental to host defense and diagnostics. The polysaccharide capsule of group B *N. meningitidis* is poorly immunogenic and thus is an important model for studying pathogen-host co-evolution through understanding the molecular basis of the host immune response. We used a modified reverse-transcriptase PCR to amplify and sequence the V-genes of murine hybridomas produced against types B and C capsular PS. Databank analysis of the sequences encoding the V-genes of type C capsular PS mAb, 4-2-C, reveal that heavy chain alleles are recurrently used to encode this specificity in mice. Interestingly, a V-gene from the same germline family also encodes the V-domain of mAbs 2-2-B, which targets the antigenically distinct serogroup B capsular PS. Somatic mutation, junctional diversity and alternative light chains collectively impart the specificity for these serologically distinct epitopes. Knowledge of the specific immunoglobulin genes used to target common bacterial virulence factors may lead to insights on pathogen-host co-evolution, and the potential use of this information in pre-symptomatic diagnosis is discussed.

Keywords: Immunoglobulin variable region genes; Group-specific immunity; Carbohydrate antigen; Capsular polysaccharide; Recurrent response; *Neisseria meningitidis*

1. Introduction

Neisseria meningitidis is a leading cause of septicemia and meningitis, with case-fatality rate of about 10% despite the availability of effective antibiotics (Solberg, 1998). One of the major virulence factors of *N. meningitidis* is its capsular polysaccharide (PS), which also carries serological specificity for the current classification of this organism into different serogroups (Knapp and Koumans, 1999). Of the 13 known serogroups of meningococci, 5 (serogroups A, B, C, Y, and W135) are responsible for causing most of the observed disease (Pollard and Levin, 2000). In North America and Europe,

Abbreviations: H3, CDR3 of heavy chain variable region; V-gene, immunoglobulin variable region gene

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Table 1
Chemical composition of the capsular polysaccharides in *Neisseria* spp. (8.9)

Serogroup	Structural repeating unit
B (homopolymer)	α NeuNAc--(2--->8)--NeuNAc
C (homopolymer)	α NeuNAc--(2--->9)--NeuNAc 7 8 7 8 ⋮ ⋮ ⋮ ⋮ OAc OAc OAc OAc

most of the meningococcal diseases are due to organisms of serogroups B, C, Y, and W135 (Pollard et al., 2001), which share the similarity of having sialic acid as a component in their capsules. Sialic acid components are found in eukaryotic tissues as important cell surface molecules, but when present in prokaryotic cells, they are often associated with virulence.

The capsules of serogroups B and C meningococci are made up of homopolymers of sialic acids linked through alpha 2,8 (for serogroup B) or alpha 2,9 linkages (in serogroup C) (Table 1) (Bhattacharjee et al., 1975). In serogroups Y and W135, capsules are comprised of heteropolymers of disaccharides of sialic acid linked with glucose (serogroup Y) or with galactose (serogroup W135) (Bhattacharjee et al., 1976). Besides sharing similar chemical structures, the genetics of the biosynthesis of these sialic acid containing capsules are also very similar. Strains B, C, Y, and W135, share a similar capsular polysaccharide synthesis operon, which differ from one another in only the polysialyltransferase gene (*siaD*) (Claus et al., 1997). The capsular polysaccharides have undergone antigenic variation, presumably due to selection pressures from host antibody responses, despite similarities in the genetics of capsule biosynthesis and chemical structure. Therefore, serological specificities exist, and both polyclonal and monoclonal antibodies (mAbs) are able to distinguish and identify four serogroups of meningococci as distinct entities. Antibodies against the PS antigens of a number of bacterial disease agents including *N. meningitidis* have been shown to have microbicidal activities which subsequently lead to the development of effective active vaccines for the induction of protective serum antibodies (Kilpi et al., 2003; Lindberg, 1999a; Vermont et al., 2003; Zangwill et al., 2003). PS immunogens are typically T-cell-independent B-cell antigens. The immunogenicity of carbohydrate vaccines is enhanced by conjugating them to protein components, which can also trigger B cells in a T-cell-dependent fashion (Lindberg, 1999b). This property is important for vaccines that are required to produce long lasting protection as T-dependent responses are required in order to drive affinity maturation and drive B cells into the memory pool (Vinuesa et al., 2002). Acellular subunit vaccines are now available for the control of infection of three of the four main serogroups (C, Y, and W135). However, a capsule vaccine for the control of serogroup B meningococci has not been successfully developed and therefore no vaccine protection exists for the B serogroup. The reasons for this may include poor immunogenicity of this component due to chemical similarity with the glycolipid antigen of the neural cell adhesion molecule (n-CAM) (Muhlenhoff et al., 1998). The immunobiology of the B serogroup capsular PS is therefore of great importance.

Increasing evidence suggests that protective immunoglobulin Variable region (V-genes) assemblies can be repeatedly expressed in the B-cell pool (Pinchuk et al., 1995; Scott et al., 1989). The host antibody response can be encoded by a restricted set of heavy chain variable region genes (V_H) to certain types of antigens, which limits the clonality of the B-cell response (Bona, 1993). This has been observed in antibody responses to Vesicular Stomatis Virus (VSV), *Haemophilus influenzae* capsular polysaccharide and has been inferred in southern analysis of the antibody response against *N. meningitidis* serogroup C capsular polysaccharide (Garcia-Ojeda et al., 2000). Antibody recurrence describes a range of similarities in antibody structures elicited to the same epitope. For example, recurrent V-gene responses may be defined as an antibody response encoded either by a predominant, recurrent, or restricted set of V-gene segments (Berry, 1999). A pattern of recurrence exists if the same or highly related antibody gene structures are produced to the same epitope in multiple individuals/animals (Kalinke et al., 1996; Kavaler et al., 1990; Mo et al., 1993; Solin et al., 1992). A B-cell response is termed a restricted response, which is an extreme case of recurrence, when the identical VK chain (Adderson et al., 1992; Akolkar et al., 1987; Casadeval et al., 1994; Mo et al., 1993; Patera et al., 1995; Scott et al., 1989), V_H chain (Pascual et al., 1992), or both (Griffiths et al., 1984; Ikematsu et al., 1998; Kaartinen et al., 1984; Liu et al., 1971) are predominantly found to encode antibodies to the same epitope of independently derived clones. All of these responses have in common an antigen with a repetitive structural organization, and/or induce a co-comittant T-cell-independent B-cell response, as they do not require mature T cells to elicit an antibody response. We speculate that stringent and or frequent host-pathogen co-evolution may select for recurrent antibody responses.

In order to examine immunoglobulin variable region gene usage in response to antigenically variant epitopes on capsular antigens of *N. meningitidis* we cloned and sequenced the cDNA of rearranged heavy and light chain variable domains of two anti-meningococcal monoclonal antibodies produced in mice against serogroups B and C capsular PS. We compared the specificity of these mAbs in relation to V-gene usage and compared these to other monoclonals in available databanks. These data reveal that somatic variation of the same V_H gene, as well as the use of alternative light chains, contribute to specificity for PS of two different serogroups of meningococci. The results of the analysis of these V-genes in relation to other serogroup C PS-specific mAbs, developed independently by others laboratories, are presented here.

Table 2
Summary of relevant properties of the V_H and V_L regions of murine mAbs specific for *N. meningitidis* capsular PS

Clone	Class	Serogroup specificity	GenBank accession number	Reference(s)
2-2-B	M/κ	B	V_H AY639151 V_L AY639150	This study
4-2-C	G3/κ	C	V_H AY639149 V_L AY639148	This study
C2/655.7	G1/κ	C	V_H AY310523 V_L AY310524	Garcia-Ojeda et al. (2000, 2003)
177.16	G1/κ	C	V_H AY229941 V_L AY229942	Garcia-Ojeda et al. (2000, 2003)
2016.3	G3/κ	C	V_H AY229955 V_L AY229956	Garcia-Ojeda et al. (2000, 2003)

2. Materials and methods

2.1. Hybridoma cell culture

The hybridomas relevant to this paper are listed in Table 2. The hybridoma cell lines 7H9-4 and 5C1-3H7 2-2-B secrete mAbs 4-2-C and 2-2-B to *N. meningitidis* and are specific to the serogroups C and B sialic acid containing capsular polysaccharides, respectively. These lines were produced through the fusion of immune splenocytes of mice immunized with whole encapsulated organism as described previously (Mandrell and Zollinger, 1982). The cell lines were expanded in BD-cell mAbs media with 10% fetal bovine serum (FBS) and 2% hybridoma cloning factor (IGEN International, Inc., Gaithersburg, MD, USA). High density culture in Integra CL300 flasks (INTEGRA Biosciences Inc., Ijamsville, MD, USA) were used according to commercial instruction for the production of monoclonal antibody for use in ELISA. We confirmed the isotype of monoclonal antibody 2-2-B and 4-2-C using a commercial isotyping kit (Roche, Laval, QC, Canada) (Table 1). Heavy and light chain isotype determination was necessary in order to select the correct oligonucleotide primers for cloning V_H and V_L cDNAs.

2.2. Enzyme-linked immunosorbent assay on meningococcal antigens

The reaction of monoclonal antibodies with meningococcal bacteria was studied by an indirect whole cell ELISA according to the method of Abdillahi and Poolman (1987) for typing of meningococci with the following modifications: overnight incubation with the monoclonal antibodies at 4 °C, followed by detection with horseradish peroxidase-conjugated goat anti-mouse IgG $F(ab)_2$ fragment-specific antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) diluted 1:5000 in 2% BSA–PBS for incubation at room temperature for 4 h.

When testing monoclonal antibodies for their reactions with the extracted capsules, purified capsular polysaccharide from serogroup C was purchased from the National Institute of Biological Standards and Control (Potters Bar, Hertfordshire, UK), while capsule polysaccharide from serogroup B was extracted and purified by a method similar to that described by Nato et al. (1991). Capsule polysaccharide antigens were coated at a concentration of 10 μg/ml PBS at 4 °C overnight. The remainder of the procedure was similar to the indirect whole cell ELISA method described above.

2.3. RNA extraction

Approximately 1–2 million log-phase mAb-secreting hybridoma cells were collected by pelleting at $300 \times g$ and homogenized via passage through a 20-guage needle using an RNase-free 5 ml syringe. Total RNA was isolated using RNeasy® Mini Spin kits according to the manufacturer's instructions (QIAGEN®).

2.4. cDNA synthesis, polymerase chain reaction, and analysis of sequences and database searches

The RNA was reverse transcribed into cDNA, PCR amplified and cloned using methods as described previously (Barbas et al., 2001; Gubbins et al., 2004). The nucleotide and inferred amino acid sequences of 4-2-C and 2-2-B light chains were analysed for relatedness using MacVector Software (version 7.1.1). The ClustalW analysis nucleotide analysis was performed at slow speed with an open gap penalty of 10 and an extend gap penalty of 5. The amino acid sequence analysis was performed using the Blosum 30 algorithm with a gap penalty of 10 and an extend gap penalty of 0.1.

2.5. Nucleotide databank accession numbers

The sequences generated during the course of this work were submitted to GenBank database at NCBI and were given accession numbers as follows: 4-2-C V_H, AY639149; 4-2-C V_L, AY639148; and 2-2-B V_H, AY639151; and 2-2-B V_L, AY639150 (Table 2).

3. Results

We have determined the identity of the immunoglobulin V-genes encoding mAbs to groups B and C capsular PS and compared the genetic relationship with antibody functionality. These monoclonal antibodies were developed against *N. meningitidis* and have use for evaluation in immunological assays for *N. meningitidis* (Mandrell and Zollinger, 1982). Monoclonal antibodies 2-2-B and 4-2-C have exquisite specificity for groups B and C meningococci, respectively, with little cross-reactivity in ELISA (Fig. 1). Antibody 2-2-B binds

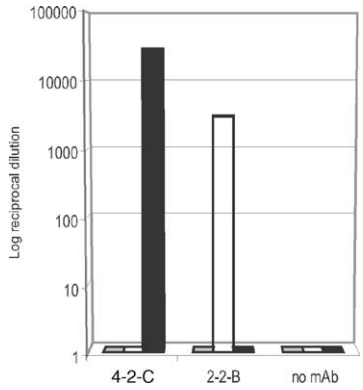


Fig. 1. ELISA reactivity of monoclonal antibodies on whole *N. meningitidis* tested in serial dilutions. This result is representative of two identical assays performed as in the material and methods. These data clearly show the exquisite type-specific reactivity of mAbs 4-2-C and 2-2-B for serogroups C and B, respectively. (Black bars, serogroup C antigen reacitivity; clear bars, serogroup B antigen reactivity; gray bars, irrelevant antigen control.)

with specificity to the type B capsular polysaccharide antigen, with the reactivity of the antibody with capsular polysaccharides from type C at background levels. In contrast, mAb 4-2-C binds with high specificity to type C capsular polysaccharide with no reaction against encapsulated serogroup B organisms. Indeed, analysis of the serogroup reactivity of these two mAbs on 13 distinct serogroups confirms the utility of these mAbs in meningococcal diagnostics (Table 3) The discrete difference between groups B and C sialic acid containing polysaccharide antigens is limited to the type of bond between the sialic acid molecules and the *O*-acetyl group in group C PS (Table 1). In this case, mAb 4-2-C is against a homopolymer of sialic acids linked in alpha 2,9 linkages which is found in the type C capsular PS, and mAb 2-2-B is against a homopolymer of sialic acid linked in alpha 2,8 linkages, found in type B capsular PS.

We determined the molecular sequence of the expressed and rearranged immunoglobulin V-genes by sequencing the cDNA corresponding to the V_H and V_L for each hybridoma clone (Figs. 2 and 3, respectively). Consensus and degenerate forward primers were designed to be complementary to sequences in the upstream leader region or the N-terminal framework 1 regions of most known murine V_H and V_L genes and back primers were based upon conserved constant region sequences (Dattamajumdar et al., 1996; Kabat et al., 1991).

Table 3
Reactions of anti-B, anti-C monoclonal antibodies against 13 known serogroups of meningococci

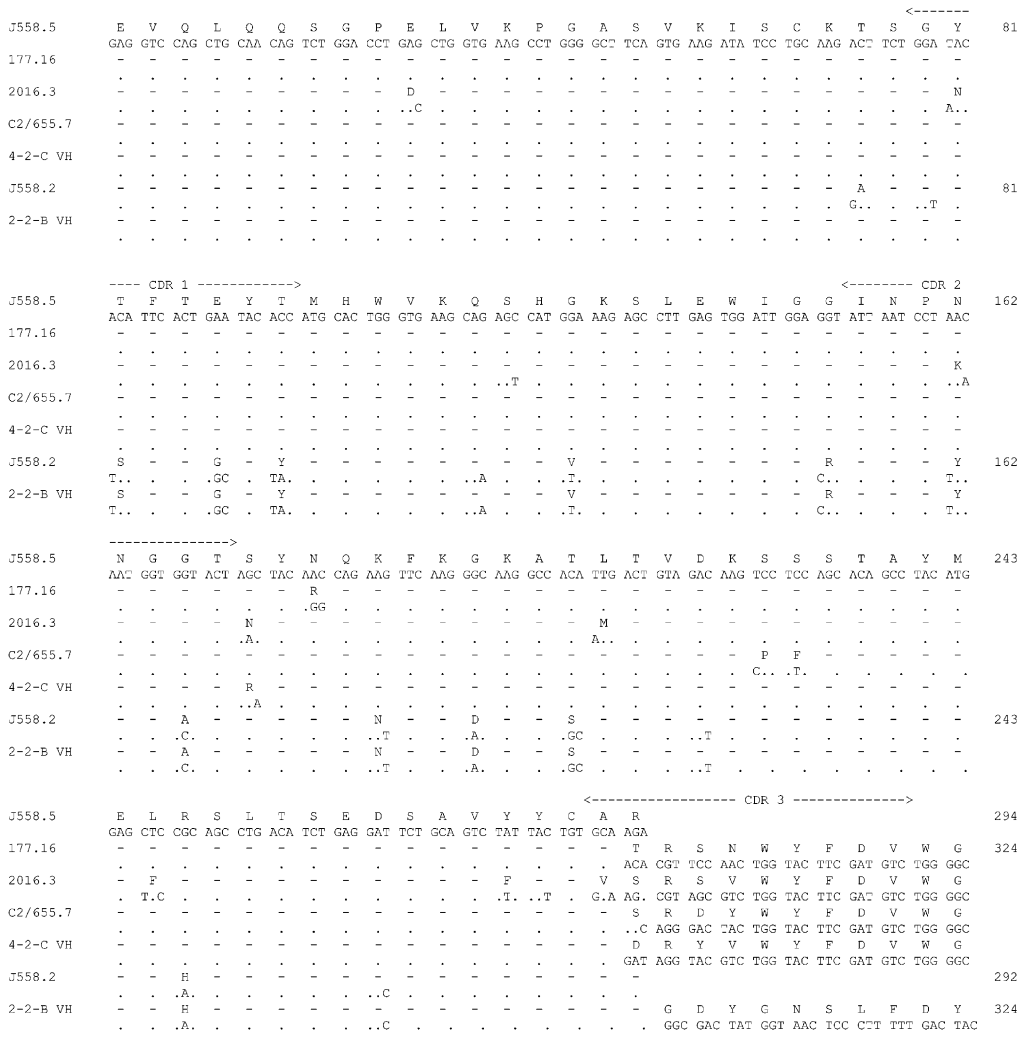
Serogroups (strain number)	ELISA OD (405 nm)	
	Anti-B 2-2-B	Anti-C 4-2-C
A (2E)	0.012	0.011
B (99M)	***0.846***	0.015
C (60E)	0.022	***3.410***
D (1613)	0.044	0.040
29E (521)	0.021	0.021
H (1890)	0.018	0.014
I (1486)	0.022	0.020
K (7811)	0.025	0.024
L (76189)	0.025	0.021
W135 (III)	0.014	0.011
X (Slaterus X)	0.012	0.012
Y (Slaterus Y)	0.020	0.021
Z (Slaterus Z)	0.026	0.025
No antigen control	0.001	0.004

Highly similar alleles from the J558 immunoglobulin V_H family encode the V_H of the anti-type B and C capsular polysaccharide mAbs. Our analysis of the cDNA sequences (Fig. 2). Analysis of 4-2-C and 2-2-B reveals that the heavy chain of 4-2-C is encoded by a J558.5 allele linked to a DH SP2.8.01 and a JH1.01 cassette, whereas 2-2-B is encoded by a J558.2 allele linked to a DH SP2.7.01 allele and a JH2.01 cassette. Interestingly, the 4-2-C V_H and 2-2-B V_H have 93% nucleotide identity through their V_H region resulting in 13 amino acid substitutions; 4 clustered in CDR1; 4 in CDR2; and the other mutations falling outside of the CDRs. The primary sequences of 4-2-C and 2-2-B are very different in the CDR3. The length of the CDR3 domain of 2-2-B V_H is 12 amino acids whereas 4-2-C has 10 amino acid residues. Interestingly, in these two V_H-domains, the CDR3 region has only 52% identity at the nucleotide level but the inferred amino acid sequence reveals that CDR3 of 4-2-C V_H has three hydrophobic amino acid residues (W, F, W) while 2-2-B H3 has one hydrophobic amino acid residues (F) (Fig. 2). These hydrophobic types of residues have been implicated as being important for antibody binding to carbohydrate antigens (Hutchins et al., 1996; Smithson et al., 1999). This is consistent with the findings of others (Garcia-Ojeda et al., 2003) who found that 23/39 (∼59%) C-type PS reactive monoclonal antibodies were likely expressing the J558 allele according to Northern blot RNA analysis. This number decreased to 50% when examining only those mAbs generated from mice immunized with the native PS antigen and excluding animals injected with protein-conjugated PS antigen conjugates.

Detailed analysis of the heavy chain domains (Fig. 2b) reveals high conservation of the CDRs in the independently derived group C reactive mAbs. This is quite remarkable given that the CDR3 is comprised of the rearrangement of three separate genetic mini-elements and occurred independently in different B cells, in different animals. The V_H of 2-2-B, the anti-group B mAb has a distinct and extended CDR3 region, but otherwise is highly identical I the CDR1 and 2 regions. To the authors knowledge this is the first time that the V-gene sequence of an anti-group B capsular PS mAb has been determined.

There is a recurrent use of identical light chain variable regions to encode monoclonal antibodies to group C PS. Remarkably, all of the anti-C PS mAbs, which were independently derived, use the identical light chain variable region (with a few changes in our sequences due to primer instilled changes). The 4-2-C light chain has very high identity with the light chain genes from other previously identified group C PS mAbs (Fig. 3). Of note, the Vk domain of 4-2-C has 100% identity with Vk domain of the 177.16 mAb further

(A)



(B)

CLONE	CDR1	CDR2	CDR3
177.16	**G** Y **T F T E Y T**	**I N P** N **N G G T**	A T **R** S N **W Y F D V**
8-8-10	GGA TAC ACA TTC ACT GAA TAC ACC	ATT AAT CCT AAC AAT GGT GGT ACT	GCA ACA CGT TCC AAC TGG TAC TTC GAT GTC
C2/655.7	**G** Y **T F T E Y T**	**I N P** N **N G G T**	A S **R** D Y **W Y F D V**
8-8-10	GGA TAC ACA TTC ACT GAA TAC ACC	ATT AAT CCT AAC AAT GGT GGT ACT	GCA AGC AGG GAC TAC TGG TAC TTC GAT GTC
2016.3	**G** N **T F T E Y T**	**I N P** K **N G G T**	V S **R** S V **W Y F D V**
8-8-10	GGA AAC ACA TTC ACT GAA TAC ACC	ATT AAT CCT AAA AAT GGT GGT ACT	GTA AGC CGT AGC GTC TGG TAC TTC GAT GTC
4-2-C	**G** Y **T F T E Y T**	**I N P** N **N G G T**	A D **R** Y V **W Y F D V**
8-8-10	GGA TAC ACA TTC ACT GAA TAC ACC	ATT AAT CCT AAC AAT GGT GGT ACT	GCA GAT AGG TAC GTC TGG TAC TTC GAT GTC
2-2-B	**G** Y S F T G **Y** Y	**I N P** Y **N G** A **T**	A **R** G D Y G N S L **F D** Y
8-8-12	GGT TAC TCA TTC ACT GGC TAC TAC	ATT AAT CCT TAC AAT GGT GCT ACT	GCA AGA GGC GAC TAT GGT AAC TCC CTT TTT GAC TAC

Fig. 2. Nucleotide and inferred amino acid sequences of V_H (heavy chain variable region) domains of anti-group C PS monoclonal antibodies. (A) Inferred amino acid sequence and nucleotide alignment of the immunoglobulin heavy chain variable regions (V_H) of anti-meningococcal polysaccharide groups B and C monoclonal antibodies. The J558.5 sequence represents the closest corresponding germline V_H sequence to 4-2-C V_H and is used as a consensus for the related group C-specific mAbs. The 177.16, C2/655.7, and 2016.3 sequences are the corresponding V_H genes from other functional group C anti-meningococcal antibodies submitted previously to NCBI by Garcia-Ojeda et al. (2003). The J558.2 sequence represents the closest corresponding germline V_H sequence to the V_H of anti-group B PS mAb 2-2-B V_H (GenBank). A dash (-) indicates the same amino acid in the clones. A single dot (·) indicates the same nucleotide codon, in cases where more the one dot is shown within a codon these represent the same nucleotide as the germline homolog immediately above the sequence. (B) Antigen contact domains (CDRs 1–3) of the inferred amino acid alignment showing identity of the heavy chain of 5 murine anti-*N. meningitis* capsular polysaccharide monoclonal antibodies. Conserved positions are depicted in bold. The hydrophobic residues of phenylalanine (F), believed to be important in PS contact are conserved in all anti-C mAbs. The hyphenated numbers under the clone names refers to the number of amino acid residues in CDR1–CDR2–CDR3, respectively.

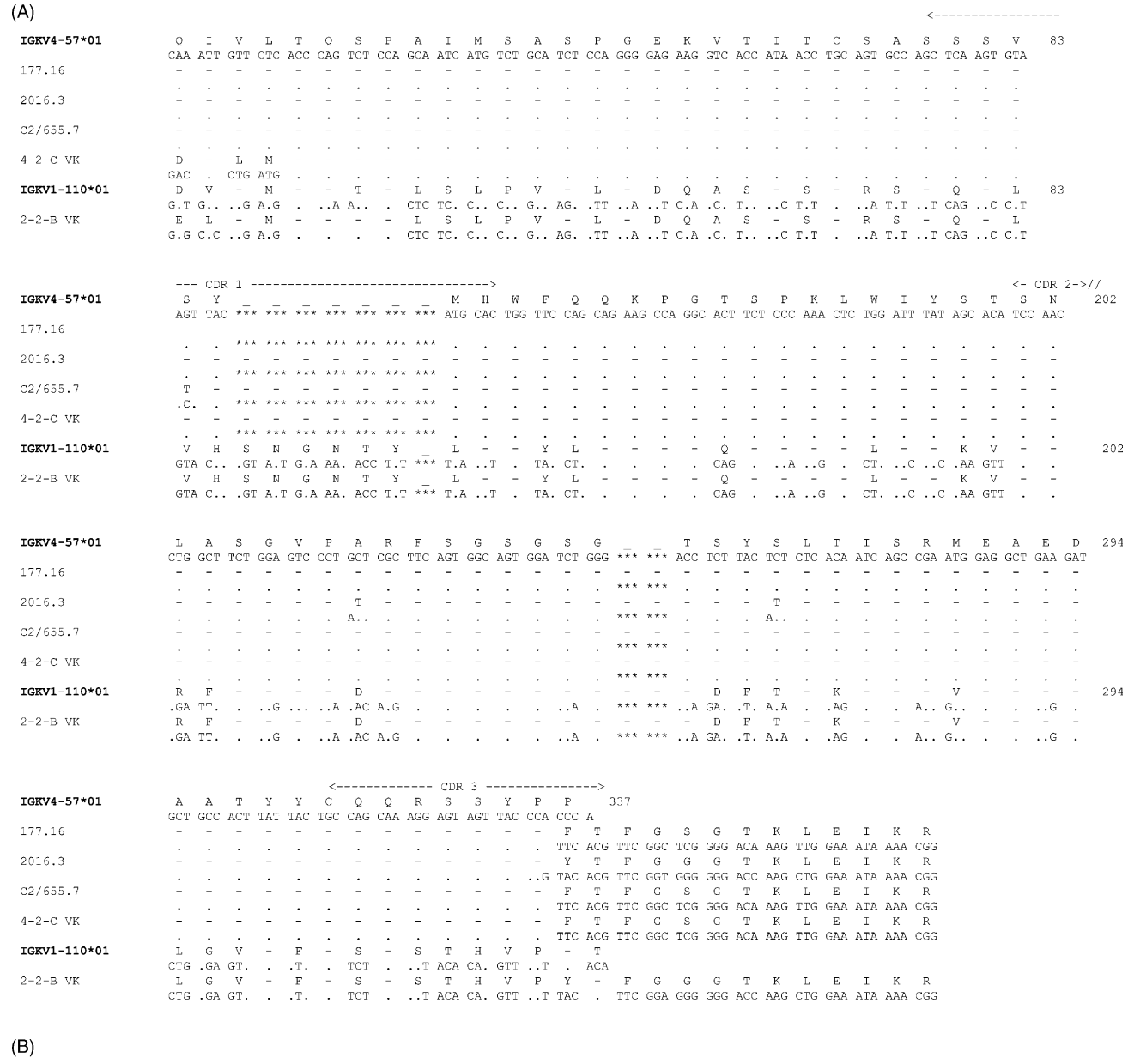


Fig. 3. Light chain variable region domains of anti-group C and B PS monoclonal antibodies. (A) Inferred amino acid sequence and nucleotide alignment of the immunoglobulin light kappa chain variable regions (V_K) of anti-meningococcal polysaccharide monoclonal antibodies. The IGKV4-57*01 sequence represents the closest corresponding germline V_K sequence to 4-2-C V_K as determined by both V-Quest (http://imgt.cines.fr) and Ig-BLAST at NCBI. The 177.16, C2/655.7, and 2016.3 V_K sequences are the corresponding V_K genes from other functional anti-meningococcal antibodies submitted previously to NCBI by Garcia-Ojeda et al. (2003). The IGKV1-110*01 sequence represents the closest corresponding germline V_K sequence to 2-2-B V_K (V-Quest). A dash (-) indicates the same amino acid in the clones, while a dot (·) indicates the same nucleotide codon in the clones. (//) Stands for the seven codon deletion in CDR2 which is not depicted. (B) Inferred amino acid sequence alignment of the anti-group B and anti-group C kappa light chain Variable region domains of clones 2-2-B and 4-2-C, respectively. A star (*) indicates the same amino acid residue identity in a position, while a dot (·) indicates a different residue, and a dash (-) indicates inserted spaces placed in the sequence to provide maximum identity. The light chains sequences have 53% identity over the region depicted.

supporting the recurrent recruitment of particular V-domains to this antigen, and suggesting an important role for the light chain in determining type C capsular PS specificity. We exclude our observed changes in the sequence of the framework 1 region of 4-2-C VK sequence, as these were templated by the specific primer used to amplify this cDNA. Clones 2016.3 and 177.16 light chain domains are encoded by a Jk2 and Jk4, respectively, whereas 4-2-C is encoded by a JK4.

Light chain variable region genes encoding anti-PS mAbs are unmutated. The light chain sequences of mAbs 4-2-C and 2-2-B are essentially pristine compared to the assigned germline Vl genes without somatic mutations in the sequence away from germline (Fig. 3). The Vk of 2-2-B has one change at the C-terminal end of in CDR3 at position 112 (P to Y), which is most likely due to junctional-joining diversity. The V-kappa sequences of these clones are distinct and this likely contributes to some of the differences in specificity for sialic acid linkages. The functional light chain sequences were also analyzed for relatedness to the available databanks.

4. Discussion

Bacterial antigenic variation elicits recurrent V_H genes with alternative light chain usage in B cells of the murine host. There is recurrent use of germline antibody gene assemblages to encode antibody binding domains to group C or B bacterial capsular polysaccharides. Comparisons of the molecular sequences of 4-2-C and 2-2-B indicate that the V_H-domains share a high degree of sequence identity (Fig. 2). Furthermore, the 4-2-C V_H and 2-2-B V_H have 99 and 100% identity at the nucleotide level with the J558.5 and J558.2 germline alleles, respectively (Fig. 2). The J558 V_H family is the largest V_H family in the mouse and has the most distinct members in the germline configuration. The J558 V_H gene family is known to be predominantly expressed in murine B cells although this varies between mouse strains (Haines et al., 2001; Yancopoulos et al., 1988). We have recently observed murine J558 alleles used to encode mAbs to distinct protein epitopes on the SARS-CoV (Gubbins et al., 2004).

Databank analysis reveals that the serogroup C-specific clones collectively have high identity in the V_H-CDR3 region at the amino acid level. Despite the use of multiple DH and JH elements to form the V_H-domains of these type C-specific mAbs, comparison of the inferred amino acid translations of the V_H-domains of the anti-type C PS clones (Fig. 2B) shows the clones 4-2-C, 177.16, C2/655.7, and 2016.3 collectively differ in amino acid sequence in just 6 positions over the CDR1, 2, and 3. Furthermore, the heavy chain CDR3 in all four of the type C-specific clones have a length of 10 amino acid residues and contain a conserved motif of: XXRXXWYFDV; despite the use of a different DH gene. In contrast, the heavy chain of clone 2-2-B, which is group B specific, is also encoded by a J558 allele, thus maintains high identity in the germline-encoded CDR1 and 2 regions. However, 2-2-B has an extended CDR3 of 12 residues and does not contain the CDR3 motif seen in mAbs to type C PS. This strongly suggests that a direct relationship between the molecular sequence of the V_H-CDR3 and the in vivo selection of B cells with this specificity exists and is consistent with the central role of CDR3 in determining the epitope specificity of many antibody molecules (Derrick et al., 1999; Xu and Davis, 2000). Moreover, this molecular recurrence suggests that this assemblage is commonly made in murine B cells, even when recombining minigene elements which express protein domains in multiple reading frames, and these B cells are frequently recruited to bacterial capsular polysaccharides. This is consistent with the immunogenetics of the murine antibody response to the *Cryptococcus neoformans* polysaccharide capsule in mice where identical V_H-CDR3 domains have been identified in independent hybridomas despite the multi-genic nature of CDR3 which also can suffer junctional as well as N region diversity (Casadevall and Scharff, 1991).

There is a recurrent use of identical light chain variable regions to encode monoclonal antibodies to group C PS. The 4-2-C light chain has very high identity with the light chains from other previously identified group C PS mAbs. Of note, the Vk domain of 4-2-C has 100% identity with Vk domain of the 177.16 mAb further supporting the recurrent recruitment of particular V-domains to this antigen, and suggesting an important role for the light chain in determining type C capsular PS specificity. We exclude our observed changes in the sequence of the framework 1 region of 4-2-C V_K sequence, as these were templated by the specific primer used to amplify this cDNA (Fig. 3). Recurrent usage of light chain V-domains in mice is consistent with V-gene usage to capsular PS in humans and in antibody responses to many other antigens (Casadeval et al., 1994; Fish and Manser, 1987; Griffiths et al., 1984; Patera et al., 1995; Scott et al., 1989). The relative absence of somatic mutations in the light chains V-genes suggests that the antibody responses were T-independent in nature.

Different light chains are used to target the B or C serogroup-specific PS (Fig. 3B). Serotype specificity to these PS antigens may be a function of the light chain variable region. Analysis reveals a 57% identity at the inferred amino acid level and a 67% identity at the nucleotide level between Vl of 2-2-B and 4-2-C. These data are consistent with the assignment of these kappa light chain alleles to different V-gene families (Fig. 3). The use of alternative light chains with a single V_H-domain to determine serotype specificity is consistent with findings on mAbs to variant strains of VSV (Senn et al., 2003). Notably, the CDR1 of 2-2-B is significantly extended compared to the CDR1 of the serogroup C light chains. Collectively, these data show that variation of the V_H-CDR3 and use of alternative light chains, can accommodate antigenic variation of bacterial PS. Recurrent availability and selection of B cells expressing germline V-genes supports the notion that evolutionary selection shapes host antibody repertoires, certainly somatically.

Independently derived mAbs to type C PS use the same V_H genes. Databank analysis reveals that the 4-2-C V_H has high identity with other independently derived mAbs developed against the T-independent capsular PS antigen of group of serogroup C (Garcia-Ojeda et al., 2003) (shown in alignment in Fig. 1). A BLAST search using the NCBI immunoglobulin database for murine immunoglobulin sequences reveals that the V_H of 4-2-C has highest identity with the V_H of mAb C2/655.7, a mAb raised against a T-cell-dependent type C PS conjugate vaccine (Garcia-Ojeda et al., 2003). The search also revealed that clones 177.16 and 2016.3, with the next highest identity with 4-2-C V_H, and these were developed using the T-cell-independent (TI) PS of serogroup C. The V_H of 4-2-C has 97, 96, and 93% identity at the nucleotide level with the V_H region of clones C2/655.7, 177.16, and 2016.3, respectively. The immunogenic stimulus used to raise 4-2-C, whole encapsulated *N. meningitidis* (Mandrell and Zollinger, 1982), is probably capable of producing both TI and T-dependent B-cell responses, so it cannot be determined if it was clearly one or the other. Comparison of the 4-2-C V_H genes with the other anti-serogroup C clones shows that 177.16 and 2016.3 use an alternative DH encoded by FL16.2.01 and JH1.01 to bind to the same epitope. These data are consistent with the findings of others, which show V-gene usage clearly depends upon inherent properties of the antigen to selecting the same recurrently assembled V-domains on the surface of a B cell (Fish and Manser, 1987). Collectively this shows a consistent recurrent recruitment of J558 alleles to bacterial polysaccharides and suggests that germline alleles from this family have a good fit for common bacterial PS.

Restricted immunoglobulin gene usage has been observed in immunoglobulins reactive to other TI-antigens such as *H. influenza* (Senn et al., 2003), *Streptococcus pneumoniae* (Shaw et al., 1995), and *C. neoformans* (Casadevall and Scharff, 1991; Pirofski et al., 1995) in mice, and to *H. influenza* in humans (Adderson et al., 1991). Similar recurrent antibody responses have been observed in monoclonal antibodies to antigens on other infectious pathogens, including VSV (Kalinke et al., 1996), Influenza A (Caton et al., 1986; Kavaler et al., 1990). These findings collectively reinforce earlier findings of restricted antibody responses in mice to simple synthetic hapten antigens such as phosphatidyl choline (Seidl et al., 1997), phenyl oxalozone (Delassus et al., 1995; Griffiths et al., 1984; Kaartinen et al., 1991), and Arsonate (Fish and Manser, 1987). In contrast, antibody responses to other antigens can be encoded by extremely diverse antibody genes (Akolkar et al., 1987; Sikder et al., 1985) although there may be less biological significance to responses against the types of haptenic-antigens used by those investigators. Recurrent antibody responses are not limited to single strains of mice as anti-oxazolone hapten antibody responses utilize the same V-genes in 10 different strains of mice independent of MHC background (Kaartinen et al., 1991). V_H gene usage to PS antigens has been most extensively studied in murine monoclonal antibodies produced in mice. Antibody responses to *C. neoformans* are encoded by a highly restricted use of V_H gene families although in contrast the murine response to different serogroups results in use of different V_H gene families (Casadevall and Scharff, 1991; Pirofski et al., 1995). A limitation of our study is the small sample size and we will examine the genetics of V-gene usage in other hybridomas to *N. meningitidis* capsular PS antigens.

The subtle structural differences in the sialic acid linkage of type B and type C capsular PS is countered by the host through changes in the antibody paratope structure encoded in alterative light chain V-genes and with differences in the CDR3 of the V_H-gene. The host counteracts antigenic variation of protective virulence determinants by somatically altering the structure of antibody molecules (Brunham et al., 1993). The role of antibody diversity mechanisms in producing protective antibodies continues to be an area of great interest. The relative role of the heavy chain CDR3 domain versus the light chain in determining overall specificity is not yet known for our clones, but will be evaluated by light chain shuffling of the V_H and V_L pairs in vitro as recombinant antibody molecules (Berry et al., 2003).

The expression of pathogen-associated V-genes may lead to new pre-symptomatic diagnostic tests. Restricted and recurrent antibody responses to common epitopes may aid in the development of new host response indicators (Berry, 1999). Detection of the up-regulation of a particular V-gene assembly known to be used against a specific bacterial, viral, or other immunogen by responding lymphocytes, would aid in pre-symptomatic diagnosis. Even before an infection takes hold, pre-symptomatic diagnostic testing may be possible by measurement of spikes of specific Ig RNA produced during a clonal expansion of lymphocytes and terminal blast differentiation. In this case, the amount of specific mRNA per B cell increases more than 100-fold (Yuan and Tucker, 1984). This spike in specific mRNA should be measurable by simple genetic tests. Prediction of V-genes used to common pathogenic structures may be useful information for the diagnosis of an infection or for identifying unknown pathogens through the response it elicits in the host.

In summary, we analyzed the V_H and V_L genes of independently derived hybridomas, which target the sialic acid containing capsular polysaccharides of serogroups C and B *N. meningitidis*. We found that while other genes can be used to produce mAbs to the group C PS (Garcia-Ojeda et al., 2000), mAbs from the murine host recurringly use the same V-gene assemblage to target the type C PS epitope. Furthermore, nearly identical V-gene alleles can be used to target antigenic variant PS epitopes, such as group B PS, through somatic variation and junctional diversity of CDR3 and with use of an alternative light chain partner. This illustrates the built-in redundancy of the host B cell repertoire against virulence determinants on a pathogen and is an example of how the host germline Ig repertoire has evolved somatic mechanisms to frequently and readily produce protective mAbs to common but distinct virulence determinants.

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