DNA SEQUENCES OF TWO BACTERICIDAL IgG2B MONOCLONAL ANTIBODIES SPECIFIC FOR CLASS 3 OUTER MEMBRANE PROTEIN OF *Neisseria meningitidis* B:4:P1.15

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SUMMARY

Many monoclonal antibodies (MAbs) had been generated for classification purposes against the porin proteins of *Neisseria meningitidis*, a Gram negative bacteria responsible for meningococcal disease. These MAbs could also be potentially important for passive serotherapy, based on their ability to mediate complement-dependent cell lysis (CDC), block the adhesion of the bacteria to endothelia, or stimulate phagocytosis. MAbs 15-1-P4 and CB-Nm.2 are mouse IgG2b that identify the class 3 outer membrane protein of *N. meningitidis*, serogroup B. Our group has previously shown that these MAbs compete for a B:385 (B:4:P1.15) serotype antigen preparation with almost identical affinity constants. However, when confronted with a *Neisseria* panel using whole-cell ELISA, their strain recognition varies, strongly indicating that they react with spatially close but nevertheless different epitopes on class 3 OMP.

In this paper we show that both MAbs have similar CDC ability for strain B:385. Due to their potential as agents for passive immunotherapy, we have cloned the variable regions of the antibodies using the polymerase chain reaction (PCR), and determined their sequence. We found that these belong to different mouse gene families, and show very low homology.

RESUMEN

Diversos anticuerpos monoclonales han sido generados contra las porinas de *Neisseria meningitidis*, con propósitos de la clasificación de estas bacterias Gram negativas, responsables de la enfermedad meningocóccica. Dichos anticuerpos pueden ser también potencialmente importantes para la seroterapia pasiva, basado esto en su capacidad de mediar la lisis celular mediada por las proteínas del complemento, en su propiedad de bloquear la adhesión de las bacterias al endotelio, o por último, estimulando la fagocitosis.

Los anticuerpos monoclonales 15-1-P4 y CB-Nm.2 son dos IgG2b de ratón que reconocen la proteína Clase 3 de la membrana externa del serogrupo B de *N. meningitidis*. Nuestro grupo ha mostrado previamente que estos anticuerpos compiten, con casi idénticas constantes de afinidad, ante una preparación de antígenos de membrana del serotipo B:385 (B:4:P1.15). Sin embargo, cuando son comparados usando un panel de diferentes cepas de *Neisseria*, usando un ELISA de células totales, su reconocimiento varía, lo cual indica que ambos anticuerpos reaccionan con diferentes epitopes, aunque estos se hallan cercanos espacialmente.

En el presente artículo se demuestra que ambos anticuerpos presentan una similar capacidad bactericida ante la cepa B:385. Debido a su potencialidad como agentes para la inmunoterapia pasiva, nosotros clonamos las regiones variables de ambos anticuerpos, usando la reacción en cadena de la polimerasa y luego se determinó la secuencia de cada una. Se encontró que pertenecen a diferentes familias génicas y que entre ellas la homología es muy baja.

INTRODUCTION

Neisseria meningitidis, the ethiologic agent of the meningococcal meningitis, is a Gram negative encapsulated diplococcus with the typical external membrane of these microorganisms (Peltola 1983). Twelve serogroups (A, B, C, H, I, K, L, X, Y, Z, 29E and W135) associated with different epidemiological patterns of the disease have been described for this specie, in correspondence with the antigenic specificity of the capsular polysaccharides (Frasch *et al.* 1976).

Other classification systems (Mandrell and Zollinger 1977; Poolman *et al.* 1980; Frasch *et al.* 1985) are mainly based on the immunogenic differences of the outer membrane proteins (OMP), defined as classes 1 to 5. The class 2 and 3 porin proteins, of which only one is produced by any single strain, are among the predominant components of the outer membrane of *N. meningitidis* (Tsai *et al.* 1981; Wolff and Stern 1991). The OMP show a large degree of

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antigenic diversity between different strains, and are used for the serotype classification of meningococcal isolates (Mandrell and Zollinger 1977).

Monoclonal antibodies (MAbs) against OMP have been essential for the classification of *N. meningitidis* and the study of antigenic strain variations (Zollinger *et al.* 1984; Wedege *et al.* 1991). In this paper we show that mouse MAbs 15-1-P4 (Wedege *et al.* 1991) and CB-Nm.2 (Cruz et al. 1993), specific for class 3 OMP of *N. meningitidis*, serogroup B, mediate complement-dependent cell lysis (CDC) *in vitro*. We also report the nucleotide and aminoacid sequences of the variable regions for both MAbs.

MATERIALS AND METHODS

Cell Lines

Two mouse hybridoma cell lines were used: 5DC4, that secretes the IgG2b MAb 15-1-P4 (Wedege et al. 1991) and CB-Nm.2 (Cruz *et al.* 1993), that produces a MAb with identical isotype. Both MAbs recognize the class 3 OMP of *N. meningitidis*, serotype 4. Cells were grown in RPMI-1640 (GIBCO) with 10% newborn calf serum (Cubavet), and supplemented with 2 mM glutamine, 50 uM 2-mercaptoethanol, 0.48 mM sodium pyruvate, 0.17 uM bovine insulin, and 1.3 mM cis-oxaloacetic acid (all from GIBCO).

Bacterial Strains

N. meningitidis strain B:385 (B4:P1.15), a patient isolate representative of strains circulating in Cuba, was kindly provided by Dr. Gustavo Sierra (Carlos J. Finlay Institute of Sera and Vaccines, Havana).

Bactericidal Assay

The bactericidal assay was performed according with the protocol described elsewhere (Larrick *et al.* 1990). Briefly, the antibodies were precipitated from culture supernatants with 50% $(NH_4)_2SO_4$ for 2 hours at 4°C, washed with the same solution and extensively dialyzed in phosphate buffered saline (PBS). A pool of rabbit sera (3-4 weeks of age) was used as complement source. Bacteria were adjusted to a final proportion of 4 bacteria/ul (1 OD = $3x10^9$ bacteria/ml), and a suitable set of antibody dilutions (maximum 1:640) were mixed with active complement, Gey Balanced Salt Solution, and bacteria in a 1:1:2:1 proportion, for a final volume of 125 ul. The samples were incubated in microtiter well plates (NUNC) at 37°C for 1 hour, and 25 ul of the content of each well was finally plated in triplicate Mueller-Hinton agar plates and further incubated at 37°C for 18 hours. A bactericidal titer is the dilution of antibody that reduces the number of colonies by 50% or more, with respect to the 3 types of negative controls used (an irrelevant mouse MAb, heat-inactivated complement, or no antibodies with active complement).

Oligonucleotide Primer Design

Table I shows the structure of the primers used in our experiments for the PCR cloning of the mouse immunoglobulin light and heavy chain variable region genes (Larrick *et al.* 1990; Gavilondo *et al.* 1990; Ayala *et al.* 1990; Coloma et al. 1991).

Table 1
Structure of primers for the specific amplification of light and heavy chain variable regions genes from mouse hybridoma cells

Mouse Kappa Light Chains

5'End: Framework one region (aminoacids 1 to 8).-
EcoRI/FR1-ML:
5'--GGGAATTCGA(CT)ATTGTG(AC)T(AG)AC(CA)CA(AG)(GT)(AC)TCCA-- 3'

3'End: Kappa Constant Region (amino acids 116 to 122).-
SalI/CONST-ML:
5'--GGGTCGACACTGGATGGTGGGAAGATGGA-- 3'

Mouse Gamma Heavy Chains

5'End: Framework One Region (aminoacids 1 to 7).-
EcoRI/FR1-MH :
5'--GGGAATTC(GC)AGGT(CG)(AC)A(AG)CTGCAG(CG)AGTCT-- 3'

3'End: Gamma Constant Region (amino acids 121 to 131).-
SalI/CONST-MH :
5'--GGGTCGACA(TC)CTCCACACACAGG(AG)(GA)CCAGTGGATAGAC-- 3'

Notes: Bases in parentheses are substitutions at a given position; i.e. (CT) indicates that C and T were present in equimolar amounts during the synthesis. Aminoacid positions were classified after Kabat *et al.*, 1991.

The 5' primers were constructed from information available on the conserved sequences of the first framework (FR1) regions, plus an EcoRI restriction site for cloning. The 3' primers annealed in the Ck or CH1 domains of the constant region of the light and gamma heavy chains, respectively, and had a SalI restriction site for cloning. The oligonucleotide mixtures were synthesized on an Applied Biosystems 341 PCR-Mate. No purification was performed prior to use.

RNA Extraction

This step was performed according with the guanidinium thiocyanate/cesium chloride procedure, as described (Larrick *et al.* 1989). Briefly, 10^5 cells were lysed overnight in 100 ul of guanidinium thiocyanate 4 M solution, layered over 100 ul 5.7 M CsCl solution and centrifuged at 95,000 rpm during 2 h in a TLA-100 rotor on a Beckman TL-100 bench top ultracentrifuge. The pelleted RNA was resuspended in 100 ul of diethylpirocarbonate (DEPC) treated water and potassium acetate/cold ethanol precipitated overnight at -20°C. The precipitate was washed with 80% ethanol, dried in a speed-vacuum centrifuge, and resuspended in 15 ul of DEPC-treated water.

cDNA Synthesis

First strand DNA synthesis was performed using the Boehringer-Mannheim cDNA kit; briefly, total RNA samples (approximately 0.5 ug; derived from 10^5 hybridoma cells) were heated at 65°C for 1-5 minutes, and incubated with a mixture of RNase inhibitor, deoxynucleotides, oligo $(dT)_{15}$ as primer, and AMV reverse transcriptase, for 60 minutes at 42°C.

Polymerase Chain Reaction (PCR)

Eighty ul of PCR mix was added to the 10 ul of first strand cDNA. The PCR mix was made following the instructions of the Perkin-Elmer Cetus PCR kit. Five ul of each oligo was added to

give a final primer concentration of 1 uM and the mixture was subjected to PCR amplification using a Perkin-Elmer thermal cycler set, for 30 cycles. The temperatures and times used for PCR were: melting at 94°C, 1 minute; primer annealing at 50°C, 1 minute; primer extension at 72°C, 1 or 2 minutes. Normally one minute ramp times were used between these temperatures. Ethidium bromide stained 2% agarose (NuSieve) gels were used to visualize PCR fragments, and 3% low melting temperature agarose (FMC) gels to isolate the desired bands.

DNA Cloning and Sequencing Reactions

Double strand PCR products obtained from low melting temperature agarose gels, and M13 mp18/19 phage sequencing vectors (Pharmacia), were digested with EcoRI and SalI (BRL) and ligated using T4-DNA ligase (Boehringer-Mannheim) at 1 U/ul in 10 ul for 10 hours at 12-13°C. Single strand DNA from phage cultures, using *E. coli* JM-101 as host cell line, were used as templates for sequence reactions. Dideoxynucleotide chain termination was carried out using ^{35} S and the Sequenase 2.0 reaction kit (USB), according to the manufacturers' instructions. At least three independent M13 clones were used to validate each sequence.

RESULTS AND DISCUSSION

MAb 15-1-P4 (Wedege et al. 1991) was generated injecting mice with live meningococci from a serogroup B strain isolated in Miami. On the other hand, CB-Nm.2 was produced immunizing with a Serotype Antigens (STA) preparation from a case-isolate (B385) strain of *N. meningitidis* (B4:P1.15), from Havana (Cruz *et al.* 1993).

Both MAbs share the same immunoglobulin subclass (IgG2b), and recognize the class 3 OMP of *N. meningitidis* in western blot (Cruz *et al.* 1993). These same authors have shown that these MAbs recognize the STA from *N. meningitidis* B385 in ELISA, as well as the intact bacteria, and that the antibodies compete with similar affinity constant for the same antigenic determinant in ELISA.

Table 2
Complement-dependent cell lysisof N. meningitidis B:385 by MAbs 15-1-P4 and CB-Nm.2

MAb Dilutions	1:20	1:40	1:80	1:160	1:320	1:640
15-1-P4	0	0	1	0	2	2
Control with inactive complement	: 31					
Control with an irrelevant MAb (1:40)	: 38					
Control without antibody	: 43					
CB-Nm.2	0	1	0	0	0	3
Control with inactive complement	: 25					
Control with an irrelevant MAb (1:40)	: 36					
Control without antibody	: 31					

Note: numbers represent the amount of colonies

We found that both MAbs have similar bactericidal ability *in vitro* against B:385, using a complement dependent assay, and a spectrum of dilutions of culture supernatants (table 2). The bactericidal activity of CB.Nm.2 and 15-1-P4 could be abolished by heat-inactivating the rabbit complement.

However, the extent of the practical application of these MAbs probably vary, in dependence of their differences in strain recognition, as shown in whole-cell ELISA experiments (table 3, with permission from Cruz *et al.* 1993). This suggests that the two MAbs may react with spatially close but nevertheless different epitopes on class 3 OMP, that could be strain-dependent.

The distinct epitope recognition exhibited by these MAbs could have its origin in the use of different serogroup B isolates for immunization, due to the disimilar nature of the employed antigens, or a combination of both.

As MAbs against OMP from strains belonging to this and other serotypes have been shown to protect animal models (Larrick *et al.* 1990), CB-Nm.2 and 15-1-P4 may have therapeutic potential as agents for passive immunotherapy in *N. meningitidis* infections. However, the use of murine MAbs in humans has been accompanied by a human anti-murine antibody response (HAMA) (Schroff et al. 1985; Shawler, et al. 1985) in roughly half of the patients. Recombinant

Table 3
Whole-cell ELISA Recognition Pattern of Monoclonal Antibodies CB-Nm.2 and 15-1-P4, for *Neisseria* strains

Positive for both antibodies:

B:4:P1.1, B:4:p1.15, B:4:P1.16, B:4:NT,
B:4,B:4P1.10, B:15:P1.16, 29E, MneX

Positive for CB-Nm.2 and negative for 15-1 P4:

B:1.1, B:5:P1.1, B:12, B:12:P1.16, Nmez, NmeL

Negative for CB-Nm.2 and positive for 15-1-P4:

B:15:P1.15, B:18:P1.6, B:19:P1.6, NT.P.1.6,
NmeA, B:8

Negative for both antibodies:

B:15, B:6, B:6:P1.6, NemH, B.catarrilis, N.subflava

```
1<---------------------------FR1-----------------------------
GAG GTG AAA CTG CAG GAG TCA GGA CCT GGC CTG GTG GCG CCC TCA CAG
glu VAL lys LEU gln glu SER GLY PRO gly LEU VAL ala PRO SER GLN

---------------------------------------------------->30  31<----
AGC CTG TCC ATT ACC TGC ACT GTC TCT GGG TTC TCA TTA ATT  ACT TCT
SER LEU SER ILE THR CYS THR VAL SER GLY PHE SER LEU ile  thr ser

-CDR1----35---a-->b   <-----------------------FR2---------------
GGT ATG GGT GTG AGC   TGG ATT CGT CAG CCT TCA GGA AAG GGT CTG GAG
gly met gly val ser   TRP ile ARG GLN pro ser GLY LYS GLY LEU GLU
-------->49   <------------------------CDR2----------------------
TGG CTG GCA   CAC ATT TAC TGG GAT GAT GAC AAG CGC TAT AAC CCA TCC
TRP LEU ala   his ILE tyr trp asp asp asp lys arg tyr asn pro ser

-------->65   <---------------------------------74---------------
CTG AAG AGC   CGG CTC ACA ATC TCC AAG GAT ACC TCC AGA AAC CAG GTA
leu lys SER   ARG LEU thr ILE ser LYS ASP thr SER arg asn GLN VAL

----FR3----------82--a---b---c----------------------------------
TTC CTC AAG ATC ACC AGT GTG GAC ACT GCA GAT ACT GCC ACA TAC TAC
PHE leu LYS ile thr SER val asp thr ala ASP THR ALA thr TYR TYR

---------->   95<-----CDR3--------100--a---b---c---d---e---f---g-
TGT GCT CGA   AAG CAT GGA GGA CTA CGA CGA GGC CGG TAC TAT CCT ATG
CYS ALA arg   arg his gly gly leu arg arg gly arg tyr tyr pro met

------>   103----------FR4-----------------------113    -CH1-->
GCC TAC   TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA    GCC AAA
ala tyr   TRP GLY GLU GLY THR ser val thr val ser ser    ala lys
```

Fig. 1 Sequence for the heavy chain variable region of 15-1-P4. FRs, CDRs, and conserved aminoacids (upper case and bold) are indicated. It can be classified as belonging to Subgroup Ib according to Kabat's database.

```
1<-----------------FR1---------------------------------------
GAT ATC CAG ATG ACC CAG TCT CCA GCC ATC CTG TCT GTG AGT CCA GGA
asp ile gln met THR GLN ser pro ALA ile leu ser val ser pro GLY

------------------------>23   <-------------CDR1-------------
GAA AGA GTC AGT TTC TCC TGC   AGG GCC AGT CAG AGC ATT GGC ACA
glu arg val ser phe ser CYS   arg ALA SER gln ser ile gly thr

-------->34   35<----------38---------------FR2--------------
AGC ATA CAC    TGG TAT CAA CAA AGA ACA AAT GGT TCT CCA AGG CTT
ser ile his   TRP tyr GLN gln arg thr asn gly ser pro arg leu

-47------->   50<-------CDR2----------->   57<----------------
CTC ATA AAG   TAT GCT TCT GAG TCT ATC TCT   GGG ATC CCT TCC AGG
LEU ile lys   tyr ala ser glu ser ile ser   GLY ile PRO ser ARG

---------FR3----------------69-----------------------------------
TTT AGT GGC AGT GGA TCA GGG ACA GAT TTT ACT CTT ACC ATC AAC AGT
PHE ser GLY SER gly SER GLY thr asp phe thr leu thr ILE asn ser

----------------82--------------------->88   <-----CDR3-----
GTG GAG TCT GAA GAT ATT GCA GAT TAT TAC TGT   CAA CAA AGT AAT
val glu ser glu ASP ile ala asp TYR tyr CYS   gln gln ser asn

---------95------->   98<---------101------FR4----------->107
AGC TGG CCG CTC ACG   TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA
ser trp pro leu THR   PHE GLY ala GLY THR LYS LEU GLU lys LYS

C kappa
-------->
CGG GCT
arg ala
```

Fig. 2 Sequence for the light chain variable region of 15-1-P4. FRs, CDRs, and conserved aminoacids (upper case and bold) are indicated. It can be classified as belonging to Subgroup V according to Kabat's database.

DNA technology can now be used to engineer chimeric murine/human monoclonal antibodies (Williams 1988; Morrison and Oi 1989; Winter and Milstein 1991; Larrick and Fry 1991; Borrebaeck 1991) in the hope that replacement of all but the murine variable (V) regions or complementarity determining regions (CDRs) with human sequences will reduce the HAMA response.

```
1---------------------------FR1---------------------------------
CAG GTT  CAC CTG CAG CAG TCT GGG GCT GAG CTG GTG AAG CCT GGG CGC
gln VAL  his LEU gln gln ser GLY ala glu LEU VAL lys PRO GLY arg

---------------------22---FR1--------------------->  30--CDR1---
TCA GTG AAG ATG TCC TGC AAG GCT TTT GGC TAC ACC TTC  TCT GCC TTT
SER VAL lys MET SER CYS LYS ALA phe GLY TYR thr PHE  ser ala phe

---------->  -36---------------------FR2------------------------
CCA ATA GAG  TGG ATG AAG CAG AGT CAT GGG AAG AGC CTG GAG TGG ATT
pro ile glu  TRP met lys gln ser his gly lys ser LEU GLU TRP ile

-->  -50---------------------CDR2-----------------------------
GGA  AAT TTT CAT CCT TAC AAT GAT GAT ACT AAA TAC AAT GAA AAA TTC
GLY  asn phe his pro tyr asn asp asp thr lys tyr asn glu lys phe

------>  -66---------------------FR3---------------------------
AAG GAC  AGG GCC AAA TTG ACT GTG GAA ACA TCC TCT AGC ACC CTG TAG
lys asp  arg ALA lys LEU THR val glu arg ser SER ser THR val TYR

-------------------------------FR3--------------------------------
TTG GAG CTC AGC CGA TTA ACA TCT GAT GAC TCT GCT GTT TAT TAT TGT
leu glu LEU ser arg LEU THR SER asp ASP SER ALA VAL TYR tyr CYS

------>  -95---------CDR3----100---a------->  103---FR4------
GCA AGG  GCC TAT TAT GGT AAC TAC TTT GAC TAC  TGG GGC CAA GGC
ala arg  ALA tyr tyr gly ASN tyr phe asp tyr  TRP gly gln gly

-----------------------113    -CH1-->
GCC ACT CTC ACA GTC TCC TCA    GCC AAA
ala thr leu thr val ser ser    ala lys
```

Fig. 3 Sequence for the heavy chain variable region of CB-Nm.2. FRs, CDRs, and conserved aminoacids (upper case and bold) are indicated. It can be classified as belonging to Subgroup IIb according to Kabat's database.

```
1---------------------------FR1------------------------------
GAC ATT GTG ATG TCA CAG TCT CCA TCC TCC CTG GCT GTT TCA GCA GGA
asp ile val MET ser GLN ser pro ser ser leu ala val ser ala GLY

--------FR1----------22--->    24----------27---a---b---c---d--e
GAG AAG GTC ACT ATG AGC TGC  AAA TCC AGT CAG AGT CTA CGC AAC AGT
glu lys val thr met ser CYS  lys ser SER gln ser LEU arg asn SER

--f--28-----CDR1-------------->  -35--------FR2-----------------
AGA ACC CGG AGG AAC CAC TTG GCT  TGG TAC CAG CAG AAA CCA GGG CAG
arg thr arg arg asn his LEU ala  TRP tyr GLN gln lys pro gly gln

--------FR2--------------->  -50------CDR2------------->   -57
TCT CCT AAA TTG CTG ATC TAC  TGG GCA TCC ACT AGG GAA TCT   GGG
ser pro lys leu LEU ile tyr  trp ala ser THR arg glu ser   GLY

----------------------FR3----------------------------------------
GTC CCT GAT CGC TTC ACA GGC AGT GGA TCT GGG ACC GAT TTC ACT CTC
val PRO asp ARG PHE thr GLY SER gly SER GLY thr asp phe thr leu

------------------------FR3--------------------------87--->  ---
ACC ATC AGC AGT GTG CAG GCT GAA GAC CTG GCA GTT TAT TAC TGC  AAG
thr ILE ser ser val gln ALA GLU ASP leu ala val TYR tyr CYS  lys

----------CDR3------------>  ---------------FR4--------------106
CAA TCT TCT AAT CTT CGG ACG  TTC GGT GGA GGC ACC AGG CTG GAA ATC
gln ser ser asn leu arg thr  PHE GLY gly GLY THR arg LEU GLU ile
-->   --C Kappa->
AAA   CGG GCT GAT
LYS   arg ala asp
```

Fig. 4 Sequence for the light chain variable region of CB-Nm.2. FRs, CDRs, and conserved aminoacids (upper case and bold) are indicated. It can be classified as belonging to Subgroup V according to Kabat's database.

Recently, we (Larrick *et al.* 1989; Larrick *et al.* 1990; Gavilondo *et al.* 1990; Ayala *et al.* 1990; Coloma et al. 1991; Ayala *et al.* 1992) and others (Sastry *et al.* 1989; Ward *et al.* 1989) have reported the use of PCR and specific oligonucleotides for the rapid cloning and sequencing of human and mouse immunoglobulin V-regions, a basic step to proceed with further

genetic expression manipulations. These methods were successfully applied with hybridomas CB-Nm.2, and 5DC4.

The V-regions were amplified using 5'end framework one and 3'end constant region oligonucleotides, previously tested with other hybridomas. The PCR amplifications obtained for the light and heavy chain V-regions of 15-1-P4 and CB-Nm.2, were according with the expected bands size (ca.400 bp).

The complete nucleotide sequences of the V-regions were derived from the PCR amplified DNA bands cloned in M13 phage vectors. Important differences were found when the base and aminoacid composition of the V-regions of the two antibodies were compared (figures 1 to 4). Poor base homology was found in the framework and hypervariable domains.

Classified according to Kabat *et al.* (1991), the light chain V-regions of CB.Nm.2 and 15-1-P4 belonged to the distant subgroups I and V, respectively, and the heavy chain V-regions to subgroups Ib and IIb. The light chain V-region of CB-Nm.2 was characterized by a very long CDR1 domain (17 aminoacids versus 11 in 15-1-P4). In the heavy chains, 15-1-P4 showed a 15 aminoacid CDR3, versus 9 for CB-Nm.2.

The primers set used in this work, designed as "consensus" structures on the basis of existing databases, had been employed previously with success to amplify immunoglobulin variable genes segments coding for 7 different mouse MAbs, classified as belonging to Kabat et al. (1987) subgroups II, V, and VI (Larrick *et al.*, 1990; Gavilondo *et al.*, 1990; Ayala *et al.* 1990; Ayala *et al.* 1992), in the case of light chains, and to subgroups IIa, IIc, and "Miscelaneous" (Larrick *et al.*, 1990; Gavilondo *et al.* 1990; Ayala *et al.* 1990; Coloma *et al.* 1991; Ayala *et al.* 1992), for heavy chains.

The results reported in this article also reinforce the generic nature of these primer designs. As has been pointed out before (Larrick *et al.* 1989; Larrick *et al.* 1990) these very degenerate oligonucleotides can nevertheless prime the PCR reaction, suggesting that a number of mismatches can be tolerated. Investigating further in this subject, we have found that functional PCR primers can be designed with even more that 50% of non homologous bases with respect to the target DNA sequence, exception made of the ones that anneal in the 3'end (unpublished results).

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REFERENCES

Ayala, M.; M. J. Coloma; J. W. Larrick; J.V. Gavilondo-Cowley (1990): Cloning of the Light and Heavy Chain Variable Regions of the Anti-CD6 IOR-T1 Mouse Monoclonal Antibody Using the Polymerase Chain Reaction. *Biotecnología Aplicada* 7: 282-289.

Ayala, M.; Dueñas, M.; Santos, A.; Vázquez, J.; Menéndez, A.; Silva, A. and Gavilondo, J.V. (1992): Bacterial single-chain antibody fragments specific for carcinoembryonic antigen. *Biotechniques* 13: 790-799

Borrebaeck, C.A.K. (ed) (1991): Antibody Engineering. A Practical Guide. W.H. Freeman and Co. New York.

Coloma, M.J.; Ayala, M.; Gavilondo, J.V.; Larrick. J.W. (1991): Primer design for the cloning of immunoglobulin heavy-chain leader-variable regions from mouse hybridoma cells using PCR. *Biotechniques* 11: 152-156.

Cruz, S.; Fernández, M.E.; del Valle, J.; Nazábal, C; Ohlin, M. and Gavilondo, J. 1993: Monoclonal antibodies against P1, P3 and 31 Kd outer menbrane proteins of Neiseria meningitidis strain B:4.P1.15. (submitted to *Biotecnología Aplicada)*

Frasch, C.E.; McNelis, R.M. and Gotschlich, E.C. (1976): Strain specific variation in the protein and lypopolysaccharide composition of the group B meningococcal outer membrane. *J. Bacteriol.* 127:973-981.

Frasch, C.E.; Zollinger, W.D. and Poolman, J.T. (1985): Serotype antigens of Neisseria meningitidis and a proposed scheme for designation of serotypes. *Rev. Infect. Dis.* 7:504-510.

Gavilondo, J.; Coloma, J.M.; Vázquez, J.; Ayala, M.; Macías, A.; Fry, K.E. and Larrick, J.W. (1990): Specific amplification of rearranged immunoglobulin variable regions genes from mouse hybridoma cells. *Hybridoma* 9:407-417.

Kabat, E.A.; Wu, T.T.; Perry, H.M.; Gottesman, K.S. and Foeller, C. (eds.) (1991): Sequences of Protein of Immunological Interest. 5th. edn. Vol I. U.S. Department of Health and Human Services. Washington D.C.

Larrick, J.W.; Danielson, L.; Brenner, C.A.; Wallace, E.F.; Abrahamson, M.; Fry, K.E. and Borrebaeck, C.A.K. (1989): Polymerase chain reaction using mixed primers: cloning of human monoclonal antibody variable region genes from single hybridoma cells. *Bio/Technology* 7:934-938.

Larrick, J.W.; Coloma, J.; del Valle, J.; Fernández, M.E.; Fry, K. and Gavilondo-Cowley, J. (1990): Immunoglobulin V regions of a bactericidal anti-*Neisseria meningitidis* outer membrane protein monoclonal antibody. Scand *J. Immunol.* 32:121-128.

Larrick, J.W.; Gavilondo, J.V.; Coloma, M.J.; Fry, K.E. (1990a): Construction of recombinant therapeutic monoclonal antibodies. In: Therapeutic Monoclonal Antibodies. Eds. C.A.K. Borrebaeck and J. Larrick, Stockton Press, New York, pp 17.

Larrick, J. and Fry, K.E. (1991): Recombinant antibodies. Hum. Antibod. *Hybridomas* 2: 172-189.

Mandrell, R.E. and Zollinger, W.D. (1977): Lipopolysaccharide serotyping of Neisseria meningitidis by hemagglutination inhibition. Infect. *Immun.* **16**:471-475.

Morrison, S. and Oi, V.T. (1989): Genetically engineered antibody molecules. *Adv. Immunol.* **44**:65.

Peltola, H. (1983): Meningococcal disease: still with us. Rev. Infect. Dis. **5**:71-91.

Poolman, J.T.; Hopman, C.T.P. and Zanen, H.C. (1980): Immunochemical characterization of Neisseria meningitidis serotype antigens by immunodiffusion and SDS-PAGE immunoperoxidase techniques and the distribution of serotypes among cases and carriers. *J. Gen. Microbiol.* **116**:465-473.

Sastry, L.; Alting-Mees, M; Huse, W.D.; Short, J.M.; Sorge, J.A.; Hay, B.N.; Janda, K.D.; Benkovic, S.J. and R.A. Lerner (1989): Cloning of the immunological repertoire in Escherichia coli for generation of monoclonal catalytic antibodies: Construction of a heavy chain variable region-specific cDNA library. Proc. Natl. Acad. Sci. USA **86**: 5728-5732.

Schroff, R.; Foon, K.; Beatty, S.; Oldham, R.; Morgan, A. Jr. (1985): Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res.* **45**:879-885.

Shawler, D.L., Bartholomew, R.M.; Smith, L.M. and Dillman, R.D. (1985): Human immune response to multiple injections of murine monoclonal IgG. *J. Immunol.* **135**:1530-1535.

Tsai, C-M; Frasch, C.E. and Mocca, L.F. (1981): Five structural classes of major Outer Membrane proteins in Neiseria meningitidis. *J. of Bacteriol.* **146**:69-78.

Ward, E.S.; Güssow, D.; Griffiths A.D.; Jones, P.T. and Winter, G. (1989): Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli. *Nature* **341**: 544-546.

Wedege, E.; Caugant, D.A.; Froholm, L.O. and W.D. Zollinger (1991): Characterization of serogroup A and B strains of Neisseria meningitidis with serotype 4 and 21 monoclonal antibodies, and by multilocus enzyme electrophoresis. *J. Clin. Microbiol.* **29**:1486-1492.

Williams, G. (1988): Novel Antibody Reagents: Production and Potential. *Trends in Biotech.* **6**: 36.

Winter, G. and Milstein, C. (1991): Man-made antibodies. *Nature* **349**: 293.

Wolff, K., and A. Stern (1991): The class 3 outer membrane proteins (porB) of Neisseria meningitidis: gene sequence and homology to the gonococcal porin PIA. *FEMS Microbiol. Let.* **83**:179-186.

Zollinger, W.D.; Moran, E.E.; Connelly, H.; Mandrell, R.E. and Brandt, B. (1984): Monoclonal Antibodies to Serotype 2 and Serotype 15 Outer Membrane Proteins of *Neiseria meningitidis* and their use in serotyping. *Infect Immun.* **46**:260-266.