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Cloning and sequence analysis of cDNAs encoding the heavy and light chain variable regions of an Ab2beta anti-idiotypic monoclonal antibody possessing an internal image of cocaine

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Abstract

We report here the cloning and sequence analysis of cDNAs encoding the variable regions of an Ab2beta anti-idiotypic monoclonal antibody (K1-4c, gamma1kappa) that mimics the configuration of cocaine. The Ab2beta specifically binds to the human dopamine transporter as shown by confocal immunofluorescence microscopy. The sequence of the heavy chain complementarity-determining region 3 of K1-4c is strikingly similar to that of a monoclonal antibody (F11.2.32) specific for HIV-1 protease. Three or four amino acids in the epitope recognized by the anti-HIV-1 protease antibody are also present in the third extracellular loop of the dopamine transporter. This epitope is within the conserved region of the known transporters for dopamine, norepinephrine and serotonin in *Homo sapiens*, *Rattus norvegicus*, *Caenorhabditis elegans* and *Drosophila melanogaster*.

Keywords

Anti-idiotypic monoclonal antibody; Dopamine transporter; Cocaine; Nucleotide sequence

Cocaine addiction remains a major public health problem in industrialized countries. Up until now, efforts to find effective therapies for cocaine addiction have been unsuccessful. The addictive properties of cocaine are thought to result from inhibition of dopamine re-uptake by the dopamine transporter (DAT) in and around the synapses. By binding to the DAT, cocaine inhibits dopamine uptake and thus prolongs signaling at key brain synapses. Hydrophobicity analysis of the DAT reveals a topology of 12 putative transmembrane domains (TMs) with intracellularly oriented amino and carboxyl termini and a large *N*-glycosylated extracellular loop between TMs 3 and 4 [1,2]. Despite intensive study, the precise molecular mechanism of dopamine transport and of its inhibition by cocaine remains unclear. Recent studies of the DAT demonstrated the existence of differential sites for dopamine translocation and cocaine binding [2–4]. However, the precise binding site(s) of cocaine on the DAT has not yet been determined.

We have generated anti-idiotypic monoclonal antibodies (Ab2β), possessing the internal image of cocaine in their antigen-combining site, that elicited a specific anti-cocaine antibody response when injected into mice [5]. Several of these Ab2β inhibited dopamine uptake at levels ranging from 40 to 90% of the inhibition given by cocaine itself when tested on our neuroblastoma cells stably expressing the human DAT (hDAT) [6,7]. The different levels of inhibition seen with the various Ab2β antibodies may reflect the different ways in

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which they mimic the cocaine molecule. In view of this, we propose that the Ab2 β might be used as a powerful therapeutic tool against cocaine addiction since they compete with cocaine for its binding site but do not interfere with dopamine uptake as much as cocaine does [6]. To rule out the possibility of steric interference by the antibody molecule bound to the hDAT with the dopamine uptake process, we sought to clone the cDNA encoding the heavy (H) and light (L) chain variable (V) regions of our selected Ab2 β and generate peptides derived from their antigen-combining sites.

The concept of internal image represents an essential element of the network theory formulated by Niels Jerne [8]. The idiotype network is the inescapable consequence of the dual nature of the antibody molecule, which recognizes the antigen with its antigen-combining site (paratope) and is in turn recognized by other antibody molecules by virtue of its idiotype determinants (idiotopes). When injected into an animal, an antibody generated in response to an antigen (Ab1) induces different sets of anti-idiotypic antibodies specific for its many idiotopes. One set of anti-idiotypic antibodies (Ab2 β) recognizes the Ab1 paratope or a discrete portion of its antigen-combining site, and it is regarded as the “internal image” of the antigen. This Ab2 β due to its configuration which mimics the molecular configuration of the antigen, is capable of inducing Ab1-like Ab3 antibodies specific for the antigen, and of inhibiting the binding of the antigen to its specific antibody or Ab1. This antigenic mimicry does not necessarily depend on amino-acid sequence homology but involves similar binding interactions. In fact, antibodies can mimic molecules which are not proteins [9]. Erlanger's group developed an anti-idiotypic antibody mimicking taxol, a non-protein molecule with a molecular weight (MW) of 853.9 Daltons (Da) [10]. We developed anti-idiotypic antibodies which mimic the configuration of cocaine (MW: 339.8 Da).

Previous data obtained using our Ab2 β in dopamine uptake and inhibition assays strongly suggest the binding of Ab2 β to the hDAT [7]. Here we confirm by confocal immunofluorescence microscopy (Fig.1) the direct binding of one Ab2 β (K1-4c, D6) to mouse neuroblastoma N1E-115 cells stably expressing the hDAT. The 6C6 cell line used in this study was obtained by single cell subcloning [11] of the hDAT-transfected N1E-115 cell line generated in our laboratory [7]. Using a competitive inhibition enzyme-linked immunosorbent assay (ELISA), we ascertained that the binding of K1-4c to the hDAT was inhibited by soluble cocaine or cocaine-bovine albumin (BSA) as well as by the Ab1 anti-cocaine antibody (K1-4) used to generate the Ab2 β K1-4c; it was not inhibited by BSA or by an irrelevant antibody (anti-OVA) (Ho and Segre, unpublished data). These results demonstrated that the antigen-combining site of the Ab2 β bound to the DAT at the site of cocaine binding. The affinity constant ($K_a = 7.0 \times 10^6 \text{ M}^{-1}$) of K1-4c for the hDAT was determined by Scatchard plot [12] following the protocol described by Bator and Reading [13] with the only modification of using papain-digested K1-4c Fab fragments containing only one antigen-combining site instead of the whole antibody molecule. We then cloned the cDNA fragments which encode the variable regions of heavy (V_H) and light (V_L) chains of K1-4c and determined their nucleotide sequences. We compared the deduced amino acid sequences with those of germline V segments and with the most homologous monoclonal antibodies known to date.

A total mRNA fraction was isolated from 1×10^6 K1-4c hybridoma cells using the Quick Prep Micro mRNA Purification Kit (Amersham Pharmacia Biotech). Complementary DNA was produced from mRNA using First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech) and random hexanucleotide primers. Genes coding for the variable domains were amplified from cDNA by polymerase chain reaction (PCR) using a thermal cycler (MJ Research). MVH1-5' (5'-CTTCCGGAATTC~~S~~ARGTNMAGCTGSAGSAGTC-3') and MVH2-3' (5'-GGAAAGCTTATAGACAGATGGGGGTGTCGTTTTGGC-3') were used as primers for V_H corresponding to the mouse heavy chain framework 1 (FR1) region and

γ 1 constant region according to Kabat database (January 3, 2001 Dataset, <http://immuno.bme.nwu.edu/>) [14,15] and Wang et al. [16]. Similarly, MVL1-5' (5'-GGGAATTCCGAYATTGTGMTSACMCARWCTMCA-3') and MVL2-3' (5'-GGTAAGCTTGGATACAGTTGGTGCAGCATC-3') were used as primers for V_L corresponding to the mouse light chain FR1 and constant region. Restriction sites, which are underlined, are *Eco*RI and *Hind*III. Standard abbreviations have been used for mixed sites: K, G or T; M, A or C; N, A, C, G or T; S, C or G; R, A or G; V, A, C or G; W, A or T; Y, C or T. The cycling program started with 94°C for 10 min and continued with 30 cycles of the following thermocycling conditions: 94°C for 1 min, 45°C for 1 min and 72°C for 2 min per cycle (Taq DNA polymerase, Life Technologies). The amplified gene fragments were purified on 1% (w/v) agarose and converted to blunt and phosphorylated forms. Following a brief heat activation step, DNA fragments approximately 400bp containing V_H and V_L genes were cloned into the *Eco*RV sites of pETBlue-2 (Novagen) to yield pET.K1-4c. V_H .1 and pET.K1-4c. V_L .1, respectively. The K1-4c V_H and V_L inserts were sequenced using a 5'-primer (pETBlue2Up, 5'-GTCACGACGTTGTAAAACGACGCC-3') by the High Throughput Sequencing and Genotyping Unit at the Center for Comparative and Functional Genomics at the University of Illinois. Sequence analyses were done using the Biology Workbench 3.2 utility (<http://biology.ncsa.uiuc.edu/>), BLAST 2.0 Server (<http://www.ncbi.nlm.nih.gov:80/BLAST/>), the Wisconsin Package (Genetics Computer Group, Madison, WI) and Protein Explorer (<http://www.umass.edu/microbio/chime/explorer/>). The nucleotide sequences of the V_H and V_L and their deduced amino acid sequences are shown in Figs. 2 and 4. Comparison of the nucleotide sequences of the K1-4c heavy and light chain-specific cDNAs with the immunoglobulin genes listed in the Genbank database showed that the sequences have not been reported previously. The sequences of the V_H and V_L of Ab2 β K1-4c (D6) have been assigned the GenBank accession numbers AY028960 and AY028961, respectively.

The sequence analysis using the Kabat database (January 3, 2001 Dataset, <http://immuno.bme.nwu.edu/>) [14,15] revealed that the V_H of K1-4c belonged to the V_H XIV family, subgroup IIID. The complementarity-determining regions (CDR) were assigned using the IMGT database (<http://imgt.cines.fr:8104/>) [17]. The heavy chain CDR1 (aa 26 – 33), CDR2 (aa 51 – 57) and CDR3 (96 – 103) are located as shown in Fig. 2. Comparison of the V_H with mouse germline V_H sequences showed that the nucleotide sequence was most homologous to VH283 (GenBank accession number X00163) [18]. A total of 23 nucleotides of VH283 were changed in AY028960 (V_H) by possible somatic mutations; 12 amino acid residue changes at aa 3, 5, 31, 33, 50, 52, 56(2), 74, 76, 91, and 96 (2 in CDR1, 3 in CDR2 and 1 in CDR3 partial sequence of VH283). Interestingly, the CDR3 of the V_H bears striking homology to a monoclonal antibody (F11.2.32, PDB entry 2HRPH, GenBank accession number 2780903)[19] that recognizes HIV-1 protease (Fig. 2 & 3). It should be noted that the CDR3 of F11.2.32, which represents the antigen-combining site of F11.2.32, has been assigned to include some residues in the diversity (D) and joining (J) gene segments: RSGGIERYDGTYYVMDY [19]. For K1-4c, the corresponding sequence is I-GGITTVWGTYYVMDY. Like F11.2.32 [19], K1-4c recruits the almost identical D (except Trp instead of Asp at aa 104) and the identical long J_{H4} gene segments (GenBank accession number V00770) [14, 20] possibly to form a large protruding loop for the specificity of the antigen-combining site (Fig. 3). In fact, it has been proposed that in the anti-idiotypic network the D region may contain the internal image of the antigen [21]. Like F11.2.32, the K1-4c antigen-combining site may include several aromatic side chains due to the presence of three tyrosine residues (at aa 58, 107 and 108) and one tryptophane residue at aa 104. As observed in F11.2.32 [19], the hypervariable region of K1-4c may possibly form a hydrophobic cavity. Interestingly, the amino acid sequence Thr-Leu-Pro-Gly of the dopamine transporter located on the third extracellular loop between TMs 5 and 6 and close to TM 5 according to the current topological model [2] is almost identical to the epitope Ser-

Leu-Pro-Gly amino acid residues (P37–40) on the 10 amino-acid peptide (PDB entry 2HRPP, GenBank accession number 2780904) recognized by anti-HIV-1 protease F11.2.32 monoclonal antibody. Analysis of chimeric dopamine-norepinephrine transporters demonstrated that the regions from N-terminal through the first five TMs were essential for dopamine translocation, whereas cocaine binding was associated with TMs 6–8 [4]. In that report, Giros et al. postulated that the third extracellular loop between TMs 5 and 6 and the fourth extracellular loop between TMs 7 and 8 were the possible regions of cocaine binding. A recent study [2] of several DAT TM polar mutants revealed that substitution of the polar residue Thr (T285A) in the Thr-Leu-Pro-Gly close to TM5 reduced a cocaine analog CFT [(-)-2-beta-carbomethoxy-3-beta-(4-fluorophenyl)tropane] binding affinity to the DAT by more than 50% and produced a CFT/Dopamine affinity ratio of less than 0.5. It is well documented that the transporters for norepinephrine and serotonin, which have 60 – 70 percent amino acid sequence homology with the DAT, also serve as cocaine recognition sites and their function are also inhibited by cocaine. These transporters and the DAT are encoded by the same gene family. Sequence alignments of the known transporters for dopamine, norepinephrine and serotonin in *Homo sapiens* (GenBank accession numbers AAG33844, P23975, and X70697, respectively), *Rattus norvegicus* (GenBank accession numbers I59558, CAA73665, and X63253, respectively), *Caenorhabditis elegans* (GenBank accession number X19158 for the DAT) and *Drosophila melanogaster* (GenBank accession number AAF76882 for the DAT and GenBank accession number U04809 for the serotonin transporter) revealed that the amino acid sequence Thr-Leu-Pro-Gly or Ser-Leu-Pro-Gly (for the *Drosophila* serotonin transporter) was conserved in all of these transporter proteins.

The V_L of K1-4c belonged to the V_κ IV family, subgroup II. The light chain CDR1 (aa 27 – 36), CDR2 (aa 55 – 57) and CDR3 (94 – 101) are located as shown in Fig. 4. Comparison of the V_L with mouse germline V_κ sequences showed that the nucleotide sequence was most homologous to K1A5 (GenBank accession number D00081) [22]. A total of 10 nucleotides of K1A5 appear to have been changed in AY028961 (V_L) by possible somatic mutations, resulting in 6 amino acid residue difference at aa 2, 3, 4, 7, 33, and 90. The amino acid sequence of the light chain CDR regions, except for one amino acid (aa 33: Thr instead of Asn) in CDR1, was identical to the sequence of the germline segment K1A5. The J gene segment of V_L is identical to mouse germ line $J_{\kappa 5}$ gene segment (GeneBank accession number V00777) [23]. Since anti-idiotypic response may depend on selection of non-germ line sequences [24], it is unlikely that the light chain of K1-4c significantly contributes to the specificity of this antibody. It is worth noting that many antibodies of discrete specificities, such as anti-GAT, anti-DNP, anti-flagellin, anti-phosphorylcholine, anti-digoxin, anti-phenyloxazolone, and anti-DNA, used almost the same V_κ subgroup in association with various heavy chains [22]. Random pairing of a given V_κ chain with distinct heavy chains would probably favor an escape from selection in evolution, especially in view of the possible role of the idiotypic network acting as a “built-in” selection pressure [22].

The above observations suggest that the amino acid(s) (Ser/Thr-Leu-Pro-Gly) in the three or four positions close to TM5 of the DAT may contribute to the binding of cocaine. Further studies by point mutation or peptide mapping will be needed to confirm this prediction. We are currently generating the peptides from the antigen-combining region of K1-4c. We will examine whether indeed these peptides inhibit cocaine binding to the DAT without interfering with dopamine uptake. If this is so, these peptides may be used as novel “cocaine antagonist” peptides to study the interaction of cocaine and the dopamine transporter as well as potential therapeutic anti-cocaine addiction agents.

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Abbreviations

aa	amino acid(s)
bp	base pair(s)
cDNA	DNA complementary to RNA
CDR	complementarity-determining region(s)
DAT	dopamine transporter
hDAT	human dopamine transporter
ELISA	enzyme-linked immunosorbent assay
FR	framework region
H	heavy chain
HIV	human immunodeficiency virus
Ig	immunoglobulin
L	light chain
MW	molecular weight
nt	nucleotide(s)
PCR	polymerase chain reaction
TM	transmembrane domain
V	variable region

References

1. Giros B, el Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, Caron M. Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol Pharmacol.* 1992; 42:383–390. [PubMed: 1406597]
2. Itokawa M, Lin Z, Cai NS, Wu C, Kitayama S, Wang JB, Uhl GR. Dopamine transporter transmembrane domain polar mutants: DeltaG and DeltaDeltaG values implicate regions important for transporter functions. *Mol Pharmacol.* 2000; 57:1093–1103. [PubMed: 10825379]
3. Kitayama S, Shimada S, Xu H, Markham L, Donovan DM, Uhl GR. Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding. *Proc Natl Acad Sci U S A.* 1992; 89:7782–7785. [PubMed: 1502198]
4. Giros B, Wang YM, Suter SB, Mcleskey SB, Pifl C, Caron MG. Delineation of discrete domains for substrate, cocaine, and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. *J Biol Chem.* 1994; 269:15985–15988. [PubMed: 8206893]
5. Schabacker DS, Kirschbaum KS, Segre M. Exploring the feasibility of an anti-idiotypic cocaine vaccine: analysis of the specificity of anti-cocaine antibodies (Ab1) capable of inducing Ab2 β anti-idiotypic antibodies. *Immunology.* 2000; 100:48–56. [PubMed: 10809958]
6. Ho M, Segre M. A novel approach to the treatment of cocaine addiction: possible use of anti-idiotypic antibodies as cocaine analog peptide drugs. *The FASEB Journal.* 2000; 14:A1134.

7. Ho M, Segre M. Dopamine uptake by mouse neuroblastoma N1E-115 cells stably expressing human dopamine transporter is differentially inhibited by anti-idiotypic Ab2 β antibodies mimicking the configuration of cocaine. *Brain Res.* 2000; 872:231–235. [PubMed: 10924700]
8. Jerne NK. Towards a network theory of the immune system. *Ann Immunol (Paris)*. 1974; 125C: 373–389. [PubMed: 4142565]
9. Fields BA, Goldbaum FA, Ysern X, Poljak RJ, Mariuzza RA. Molecular basis of antigen mimicry by anti-idiotope. *Nature*. 1995; 374:739–742. [PubMed: 7536303]
10. Leu JG, Chen BX, Diamanduros AW, Erlanger BF. Idiotypic mimicry and the assembly of a supramolecular structure: an anti-idiotypic antibody that mimics taxol in its tubulin-microtubule interactions. *Proc Natl Acad Sci U S A*. 1994; 91:10690–10694. [PubMed: 7840821]
11. Ho M, Segre M. Individual and combined effects of ethanol and cocaine on the human dopamine transporter in neuronal cell lines. *Neurosci Lett*. 2001; 299:229–233. [PubMed: 11165777]
12. Scatchard G. The Attractions of proteins for small molecules and ions. *Ann NY Acad Sci*. 1949; 51:660.
13. Bator JM, Reading CL. Measurement of antibody affinity for cell surface antigens using an enzyme-linked immunosorbent assay. *J Immunol Methods*. 1989; 125:167–176. [PubMed: 2607151]
14. Kabat, EA.; Wu, TT.; Reid-Miller, M.; Perry, HM.; Gottesman, KS.; Foeller, C. Sequences of Proteins of Immunological Interest. 5. NIH; Bethesda, MD: 1991.
15. Johnson G, Wu TT. Kabat database and its applications: future directions. *Nucleic Acids Res*. 2001; 29:205–206. [PubMed: 11125092]
16. Wang Z, Raifu M, Howard M, Smith L, Hansen D, Goldsby R, Ratner D. Universal PCR amplification of mouse immunoglobulin gene variable regions: the design of degenerate primers and an assessment of the effect of DNA polymerase 3' to 5' exonuclease activity. *J Immunol Methods*. 2000; 233:167–177. [PubMed: 10648866]
17. Lefranc MP. IMGT, the international ImMunoGeneTics database. *Nucleic Acids Res*. 2001; 29:207–209. [PubMed: 11125093]
18. Ollo R, Sikorav JL, Rougeon F. Structural relationships among mouse and human immunoglobulin VH genes in the subgroup III. *Nucleic Acids Res*. 1983; 11:7887–7897. [PubMed: 6316276]
19. Lescar J, Stouracova R, Riottot MM, Chitarra V, Brynda J, Fabry M, Horejsi M, Sedlacek J, Bentley GA. Three-dimensional structure of an Fab-peptide complex: structural basis of HIV-1 protease inhibition by a monoclonal antibody. *J Mol Biol*. 1997; 267:1207–1222. [PubMed: 9150407]
20. Sakano H, Maki R, Kurosawa Y, Roeder W, Tonegawa S. Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy-chain genes. *Nature*. 1980; 286:676–683. [PubMed: 6774258]
21. Fougereau M, Corbet S, Ollier P, Rocca-Serra J, Roth C, Schiff C, Somme G, Theze J, Tonnelle C. The internal image and the structural idiotypic network (Ab1, Ab2, Ab3) in the GAT system. *Ann Inst Pasteur Immunol*. 1985; 136C:143–156. [PubMed: 3994301]
22. Corbet S, Milili M, Fougereau M, Schiff C. Two V kappa germ-line genes related to the GAT idiotypic network (Ab1 and Ab3/Ab1') account for the major subfamilies of the mouse V kappa-1 variability subgroup. *J Immunol*. 1987; 138:932–939. [PubMed: 3100621]
23. Max EE, Maizel JV, Leder P. The nucleotide sequence of a 5.5-kilobase DNA segment containing the mouse kappa immunoglobulin J and C region genes. *J Biol Chem*. 1981; 256:5116–5120. [PubMed: 6262318]
24. Sablitzky F, Rajewsky K. Molecular basis of an isogeneic anti-idiotypic response. *EMBO J*. 1984; 3:3005–3012. [PubMed: 6084592]

Fig. 1.

Confocal immunofluorescence microscopy of Ab2 β binding to hDAT-expressing neuronal cells. Cells from the mouse neuroblastoma N1E-115 cell line, stably expressing hDAT, (Clone 6C6) were fixed with 1% glutaraldehyde (A) and incubated with a cocaine anti-idiotypic (Ab2 β) monoclonal antibody (K1-4c) (B) or an irrelevant monoclonal antibody (anti-OVA) as a control (C). The cells were then incubated with PE (phycoerythrin R)-conjugated anti-mouse IgG, diluted 1:100. Confocal images were generated on an Olympus FluoView 300 confocal laser scanning system with an Olympus BX50 microscope. Bar, 50 μ m.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
K1-4c(aa)	E	V	Q	L	Q	E	S	G	G	G	L	V	K	P	G	G	S	L	
K1-4c(nt)	GAA	GTC	CAG	CTG	CAG	GAG	TCT	GGG	GGA	GGC	TTA	GTG	AAG	CCT	GGA	GGG	TCC	CTG	54
VH283(nt)	---	--G	AT-	---	GT-	---	---	---	---	---	---	---	---	---	---	---	---	---	
VH283(aa)	-	-	M	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	
F11.2.32(aa)	D	-	-	-	V	-	-	-	-	-	-	-	Q	-	-	-	-	R	

CDR1

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
K1-4c(aa)	K	L	S	C	A	A	S	G	F	T	F	S	N	Y	A	M	S	W	
K1-4c(nt)	AAA	CTC	TCC	TGT	GCA	GCC	TCT	GGA	TTC	ACT	TTC	AGT	AAC	TAT	GCC	ATG	TCT	TGG	108
VH283(nt)	---	---	---	---	---	---	---	---	---	---	---	---	-G-	---	A--	---	---	---	
VH283(aa)	-	-	-	-	-	-	-	-	-	-	-	-	S	-	T	-	-	-	
F11.2.32(aa)	-	-	-	-	-	-	-	-	-	-	-	M	R	F	G	-	H	-	

CDR2

	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	
K1-4c(aa)	V	R	Q	T	P	E	K	R	L	E	W	V	A	S	I	T	S	G	
K1-4c(nt)	GTT	CGC	CAG	ACT	CCA	GAG	AAG	AGG	CTG	GAG	TGG	GTC	GCA	TCC	ATT	ACT	AGT	GGC	162
VH283(nt)	---	---	---	---	--G	---	---	---	---	---	---	---	---	A--	---	-G-	---	--T	
VH283(aa)	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	S	-	-	
F11.2.32(aa)	-	-	-	A	-	-	-	G	-	-	-	-	-	Y	-	S	-	-	

CDR3

	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70			
K1-4c(aa)	G	S	.	T	.	Y	Y	P	D	S	V	K	G	R	F	I	S		
K1-4c(nt)	GGT	AGT	...	ACC	...	TAC	TAT	CCA	GAC	AGT	GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	212
VH283(nt)	---	G--	AAC	---	---	---	---	---	---	---	---	--T	---	---	---	---	---	---	
VH283(aa)	-	G	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
F11.2.32(aa)	S	-	R	-	I	-	-	A	-	T	-	-	-	-	-	-	-	-	

CDR4

	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	
K1-4c(aa)	R	D	N	A	R	N	I	L	Y	L	Q	M	S	S	L	R	S	E	
K1-4c(nt)	AGA	GAT	AAT	GCC	AGG	AAC	ATC	CTG	TAC	CTG	CAA	ATG	AGC	AGT	CTG	AGG	TCT	GAA	266
VH283(nt)	--C	---	---	-A-	---	-A-	---	---	---	---	---	---	---	---	---	---	--G	---	
VH283(aa)	-	-	-	K	-	N	-	-	-	-	-	-	-	-	-	-	-	-	
F11.2.32(aa)	-	-	-	P	K	-	T	-	-	-	-	-	T	-	-	-	-	-	

CDR5

	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105		
K1-4c(aa)	D	T	A	F	Y	Y	C	A	I	.	G	G	I	T	T	V	W	G	
K1-4c(nt)	GAC	ACG	GCC	TTT	TAT	TAC	TGT	GCA	ATA	...	GGC	GGT	ATA	ACT	ACG	GTA	TGG	GGG	320
VH283(nt)	---	---	--G	---	---	---	---	-G-	TA	---	---	---	---	---	---	---	---	---	
VH283(aa)	-	-	L	-	-	-	-	R	-	-	-	-	-	-	-	-	-	-	
F11.2.32(aa)	-	-	-	L	-	-	-	R	S	-	-	-	E	R	Y	D	-	-	

CDR6

	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	
K1-4c(aa)	T	Y	Y	V	M	D	Y	W	G	Q	G	T	S	V	T	V	S	S	
K1-4c(nt)	ACT	TAC	TAT	GTT	ATG	GAC	TAC	TGG	GGT	CAA	GGA	ACC	TCA	GTC	ACC	GTC	TCC	TCA	374
F11.2.32(aa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

CDR7

	124	125	126	127	128	129	130	131	132	
K1-4c(aa)	A	K	T	T	P	P	S	V	Y	
K1-4c(nt)	GCC	AAA	ACG	ACA	CCC	CCA	TCT	GTC	TAT	396
F11.2.32(aa)	-	-	-	-	-	-	-	-	-	

Fig. 2.

Nucleotide and deduced amino acid sequences of the variable region of the heavy chain of K1-4c anti-idiotypic (Ab2 β) monoclonal antibody. A dash indicates an exact match between residues; a dot indicates a gap added to the sequence. CDRs 1, 2, 3 and JH4 are shown. The sequence has been assigned GenBank accession number AY028960.

Fig. 3.

Structure alignment of the VH of K1-4c and of F11.2.32. The three-dimensional structure alignment was done using the MSA3D program in Advanced Protein Explorer. Within the variable region of the heavy chain (H), green indicates exact match between residues; light blue indicates similar match; yellow indicates difference; red indicates mismatch or insufficient sequence data. CDR 1, 2, 3: complementarity-determining region 1, 2, 3; P: peptide shown here in turquoise (PDB entry 2HRPP). Light chain can be seen in dark blue in the background.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
K1-4c(aa)	D	I	V	I	T	Q	S	P	L	S	L	P	V	S	L	G	D	Q	
K1-4c(nt)	GAC	ATT	GTG	ATC	ACA	CAA	TCT	CCA	CTC	TCC	CTG	CCT	GTC	AGT	CTT	GGA	GAT	CAA	54
K1A5(nt)	--T	G--	T--	--G	--C	---	A--	---	---	---	---	---	---	---	---	---	---	---	
K1A5(aa)	-	V	L	M	-	-	T	-	-	-	-	-	-	-	-	-	-	-	
	CDR1																		
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
K1-4c(aa)	A	S	I	S	C	R	S	S	Q	S	I	V	H	S	T	G	N	T	
K1-4c(nt)	GCC	TCC	ATC	TCT	TGC	AGA	TCT	AGT	CAG	AGC	ATT	GTA	CAT	AGT	ACT	GGA	AAC	ACC	108
K1A5(nt)	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
K1A5(aa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	
	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	
K1-4c(aa)	Y	L	E	W	Y	L	Q	K	P	G	Q	S	P	K	L	L	I	F	
K1-4c(nt)	TAT	TTA	GAA	TGG	TAC	CTG	CAG	AAA	CCA	GGC	CAG	TCT	CCA	AAG	CTC	CTG	ATC	TTC	162
K1A5(nt)	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
K1A5(aa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	CDR2																		
	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	
K1-4c(aa)	K	V	S	N	R	F	S	G	V	P	D	R	F	S	G	S	G	S	
K1-4c(nt)	AAA	GTT	TCC	AAC	CGA	TTT	TCT	GGG	GTC	CCA	GAC	AGG	TTC	AGT	GGC	AGT	GGA	TCA	216
K1A5(nt)	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
K1A5(aa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	
K1-4c(aa)	G	T	D	F	T	L	K	I	S	R	V	E	A	E	D	L	G	L	
K1-4c(nt)	GGG	ACA	GAT	TTC	ACA	CTC	AAG	ATC	AGC	AGA	GTG	GAG	GCT	GAG	GAT	CTG	GGA	CTT	270
K1A5(nt)	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
K1A5(aa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	
	CDR3																		
	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	
K1-4c(aa)	Y	Y	C	F	Q	G	S	H	V	P	L	T	F	G	A	G	T	K	
K1-4c(nt)	TAT	TAC	TGC	TTT	CAA	GGT	TCA	CAT	GTT	CCG	CTC	ACG	TTC	GGT	GCT	GGG	ACC	AAG	324
K1A5(nt)	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
K1A5(aa)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Jκ5																		
	109	110	111	112	113	114	115	116	117	118	119	120	121						
K1-4c(aa)	L	E	L	K	R	A	D	A	A	P	T	V	S						
K1-4c(nt)	CTG	GAG	CTG	AAA	CGG	GCT	GAT	GCT	GCA	CCA	ACT	GTA	TCC	363					

Fig. 4.
Nucleotide and deduced amino acid sequences of the variable region of the light chain of K1-4c anti-idiotypic (Ab2β) monoclonal antibody. A dash indicates an exact match between residues; a dot indicates a gap added to the sequence. CDRs 1, 2, 3 and Jκ5 are shown. The sequence has been assigned GenBank accession number AY028961.