Published in final edited form as:

Immunogenetics. 2013 June ; 65(6): 473–478. doi:10.1007/s00251-013-0698-5.

Cloning of a hamster anti-mouse CD79B antibody sequences and identification of a new hamster immunoglobulin lambda constant IGLC gene region

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Abstract

Anti-CD79 antibodies have been effective at targeting B cell lymphoma cells and depleting B cells in animal models. In order to engineer recombinant antibodies with additional effector functions in mice, we cloned and sequenced the full-length cDNAs of the heavy and light chain of a hamster anti-mouse CD79B antibody. Although hamster antibodies represent a unique source of monoclonal antibodies against mouse, rat, and human antigens, sequence information of hamster immunoglobulins (IG) is sparse. Here we report a new hamster (*Cricetulus migratorius*) IG lambda constant (IGLC) gene region that is most homologous to mouse IGLC2 and IGLC3.

Keywords

hamster; CD79B; antibody; immunoglobulin light lambda; IMGT Collier de Perles

Antibodies have been indispensable tools for biomedical research and are currently the most rapidly growing class of human therapeutics (Carter, 2006). Generation of recombinant antibodies and engineered antibodies with additional properties has solved many limitations of the original hybridoma technology and made them even more versatile. However, the screening of hybridomas and cloning of antibody sequences from those hybridomas remains a critical and potentially time consuming step for these applications.

Hamsters are commonly used to generate monoclonal antibodies against other rodent and human antigens because hamsters are more evolutionarily distant to mouse and rat than the two are to each other and hamster spleen cells can fuse stably with mouse myeloma cells (Sanchez-Madrid et al., 1983). Unfortunately, sequence information for hamster immunoglobulin (IG) genes is sparse. Currently, there are limited hamster IgG DNA sequences available (Alegre et al., 1995; Collins and Whitters, 1995; Gilliland et al., 1996; Mallender and Voss, 1995; Verdino et al., 2011; Whitters and Collins, 1995). Of these, only two of the heavy (IGH) chain sequences are full-length sequences including the constant (C) region. Furthermore, the isotypes of hamster IgG are not well defined. For light chains, one kappa (IGK) chain and one lambda (IGL) chain have been identified. The cloning and sequencing of the hamster IG would be greatly facilitated if the constant region sequences of different heavy chain and light chains were available.

CD79A and CD79B associate with the IG, as coreceptors, and together they constitute the B cell receptor and are necessary for its expression and function (Lefranc and Lefranc, 2001; Wienands and Engels, 2001). Antibodies against human CD79, as well as those coupled to potent cytotoxic agents, are effective at killing human B cell lymphoma cells both *in vitro* and in mouse chimeras (Polson et al., 2007). Anti-mouse CD79 mAbs have also been shown to be effective in depleting B cells and at ameliorating autoimmune disease in MRL/lpr mice (Li et al., 2008). To engineer recombinant anti-CD79 antibodies with additional effector functions in mice, we have cloned and sequenced the full-length IG heavy and light chains of an Armenian hamster (*Cricetulus migratorius*) anti-mouse CD79B reagent antibody (clone HM79-16 or HM79b) (in the absence of genomic information this antibody is designated as IgG2,λ1 by the companies which distribute it) (Koyama et al., 1997). The heavy constant (IGHC) region sequence of HM79-16 is identical to that of hamster mAb H28.710 (Collins and Whitters, 1995), which showed highest homology to mouse IGHG2A. The lambda constant (IGLC) region of HM79-16 is different from the previously published hamster IGLC of HL4E10 (Verdino et al., 2011). Phylogenetic analysis with known mammalian IGLC confirms that hamster genes are most related to rat/mouse in evolution. It also suggests that the HM79-16 IGLC is most likely the counterpart of mouse IGLC2 or IGLC3, whereas the HL4E10 IGLC is the counterpart of mouse IGLC1.

Most of the previously published sequences were obtained by screening cDNA libraries or designing degenerate primers from N-terminal protein sequences, both of which are labor intensive. One efficient way to clone cDNA sequences is to use the RACE (rapid amplification of cDNA ends) protocol (Frohman et al., 1988) when a partial sequence is known. In order to use this approach, we first attempted to amplify the constant regions of both the heavy and light chain of the hamster anti-mouse CD79B antibody (HM79-16). Primers were designed from conserved regions of available hamster C region sequences to amplify the cDNA of HM79-16. Successfully amplified fragments were sequenced and verified. From these partial sequences of the C region, nested primers were designed to amplify the 5' or 3' of the cDNA. Finally, full-length cDNA were assembled from partially overlapping sequences and verified with additional primers.

As shown in Fig. 1, the HM79-16 heavy chain comprises a 1422 base pair (bp) coding region with a 70 bp 5' UTR and a 94 bp 3' UTR. It encodes a predicted 19 amino acid (AA) leader region (L-REGION) and a mature 454 AA protein. The variable (V) region is located from nucleotides (nt) 128–424, the junction (J) region from nt 425–476, and the constant region from nt 477–1492.

IMGT/V-QUEST (Brochet et al., 2008; Giudicelli et al., 2011) was used to compare the HM79-16 variable and junction region to available human, mouse, and rat germline sequences. The HM79-16 IGHV is closest to mouse IGHV10-1*02, with an 84.3% identity at the nt level. The J region is closest to human IGHJ5*01, with an 80.4% identity at the nt level. Multiple sequence alignments of the HM79-16 IGHG with all mouse IGHG isotype regions demonstrate highest homology (79.0%) with mouse IGHG2. It should also be noted that the C region is identical to one previously published hamster C sequence (GenBank accession number U17166) (Collins and Whitters, 1995). HM79-16 IGHG is different from that of the HL4E10, which shows highest homology to mouse IGHG1*01. The V domains and C domains of HM79-16 and HL4E10 heavy chains are shown in Fig. 3 and Fig. 4, respectively.

As shown in Fig. 2, the HM79-16 light chain sequence comprises a 720 bp coding region with a 59 bp 5' UTR and a 133 bp 3' UTR. It encodes a predicted 19 AA L-REGION and a mature 220 AA protein. The V region is located from nt 17–420, the J region from nt 421–461, and the C region from nt 462–779.

The HM79-16 IGLV region is closest to human IGLV11-55*01, showing 73.5% identity at the nt level. The J region shows a similar identity of 79.4% to human IGLJ2*01, IGLJ3*01, and IGLJ3*02. The V domains of HM79-16 and HL4E10 light chains are shown in Fig. 3. The HM79-16 light chain constant region is different from previously published hamster light chain sequences (Verdino et al., 2011; Whitters and Collins, 1995). Therefore, we compared and analyzed this sequence in more detail.

Two isotypes of IG light chains, designated as kappa (IGK) and lambda (IGL), are found in most vertebrates (Lefranc and Lefranc, 2001; Lefranc and Lefranc, 2004). In mice and humans, IGK and IGL genes are located on separate chromosomes and are well characterized. Laboratory mice have three IGLV and three IGLC that are functional. Each IGLC gene has an associated upstream IGLJ gene, and the J and C genes are arranged in two clusters (Lefranc and Lefranc, 2004), IMGT Repertoire, Locus representations, <http://www.imgt.org>. The IGLC regions of HM79-16 and previously published hamster and mouse sequences were compared in a pairwise manner. The result was summarized in Table 1. Regardless of species, the HM79-16 C region had much higher homology with IGLC (about 70–80%) than IGKC (about 40%) confirming that the C region of the HM79-16 light chain is encoded by an IGLC gene.

In comparison to the recently identified hamster lambda light chain of HL4E10 (Verdino et al., 2011), the HM79-16 IGLC region shares 74.5 % identity, suggesting that it is encoded by a different IGLC gene. The IMGT Collier de Perles (Lefranc, 2011a) of the C-LAMDA for HM79-16 and HL4E10 is shown in Fig. 5. Of the three mouse IGLC genes, HM79-16 is most homologous to IGLC2 and IGLC3 (80.1% and 80.5%, respectively) (note that mouse IGLC2 and IGLC3 are 96% identical), whereas the HL4E10 IGLC shows best homology with IGLC1 (80.5%). Amino acid alignment of hamster and mouse IGLC genes also supports the same conclusion (Fig. 4). As shown in Fig. 6, phylogenetic analysis with the IGLC genes of other mammalian species including mouse, rat, rabbit and human showed that hamster sequences are most related to mouse and rat and are most distant from humans, which is consistent with the evolutionary relationship of these species.

In summary, we have cloned and determined the full-length cDNA sequences of a hamster anti-mouse CD79B antibody and have identified a new hamster IGLC gene. The cDNA sequences will be of great use for engineering recombinant anti-mouse CD79 antibodies. The identification of the new IGLC gene will help elucidate the immunogenetics of hamster IG genes and facilitate the cloning of other hamster antibodies.

Acknowledgments

We thank Dr. Robert Eisenberg for providing the HM79-16 hybridoma; and University of Chicago Comprehensive Cancer Center DNA Sequencing & Genotyping Facility for sequencing. This work was supported by NIH NIAID grant R01-AI087645 (H. Huang). Jason Perera was partially supported by NIH/NIAID Grant 2T32AI007090.

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1      TGAGGTCAGGCAGAGGACTCTAGCCCTGTCTTCCCATTCAGTGAGCAGCACTGAAAACAA
61      GACCATCAACATGGGATTGGGACTGCAGTGGGTTTTCTTTGTTGCTCTTTTAAAAGGTGT
           M G L G L Q W V F F V A L L K G V
121     CCACTGTGAGGTGCGACTTCTGGAGTCTGGTGGAGGATTAGTGAAGCCTGAGGGGTCACT
           H C//E V R L L E S G G G L V K P E G S L
181     GAAACTCTCCTGTGTGGCCTCTGGATTACCTTCAGTGACTATTTTCATGAGCTGGGTCCG
           K L S C V A S G F T F S D Y F M S W V R
241     CCAGGCTCCAGGGAAGGGCTGGAATGGGTTGCTCACATATACACTAAAAGTTATAATTA
           Q A P G K G L E W V A H I Y T K S Y N Y
301     TGCAACCTATTACTCGGGTTCGGTGAAAGGCAGATTACCATTCTCCAGAGATGATTCCTCG
           A T Y Y S G S V K G R F T I S R D D S R
361     AAGGATGCTCTACCTGCAATGAGCAACCTGAGAACTGAGGACACGGCCACTTATTACTG
           R M L Y L Q M S N L R T E D T A T Y Y C
421     TATGGACGGTAGCTACGGTTACTGGGGCCAAGGAACCATGGTCACCGTCTCTCAGCCAC
           M D G S Y G Y W G Q G T M V T V S S A T
481     AACAAACAGCCCCATCTGTCTATCCCTTGGCCCTGCCTGTGACAGCACAACAGCACCAC
           T T A P S V Y P L A P A C D S T S T
541     GGACACGGTGACCTGGGATGCCTGGTCAAGGGCTATTTCCTGAGCCGGTGACCGTAAG
           D T V T L G C L V K G Y F P E P V T V S
601     CTGGAACCTCTGGAGCCCTGACCAGCGGCGTGACACCTTCCCATCTGTCTGCATTCTGG
           W N S G A L T S G V H T F P S V L H S G
661     GCTCTACTCCCTCAGCAGCTCAGTGACTGTACCTTCCAGCACTGGCCCAAGCAGCCCAT
           L Y S L S S S V T V P S S T W P K Q P I
721     CACCTGCAACGTAGCCCCCGGCCAGCAGCAACCAAGTGGACAAGAAAATCGAGCCCGAG
           T C N V A H P A S S T K V D K K I E P R
781     AACTGATACGGATACATGTCCTAATCCACCGGATCCATGTCCCACGTGTCCAACCTCTGA
           T D T D T C P N P P D P C P T C P T P D
841     CCTCTTGGGTGGACCATCCGTCTTCATCTTCCCCCAAAGCCAAGGATGTGCTCATGAT
           L L G G P S V F I F P P K P K D V L M I
901     CTCCTGACCCCCAAGATCACATGTGTGGTGGTGGACGTGAGCGAGAGGAGCCAGACGT
           S L T P K I T C V V V D V S E E E P D V
961     CCAGTTCAACTGGTATGTGAACAACGTAGAAGACAAGACAGCTCAGACAGAGACCCGGCA
           Q F N W Y V N N V E D K T A Q T E T R Q
1021    GCGGCAGTACAACAGCACCTACCGCGTGGTCAGCGTCTCCCCATCAAGCACCAGGACTG
           R Q Y N S T Y R V V S V L P I K H Q D W
1081    GATGAGTGGCAAGGTGTTCAAATGCAAGGTCAACAACAATGCCCTCCCTAGCCCCATTGA
           M S G K V F K C K V N N N A L P S P I E
1141    GAAAACCATCTCCAACCCAGAGGGCAAGTCCGGGTACCACAGATATATACCTTTCTCTCC
           K T I S K P R G Q V R V P Q I Y T F P P
1201    GCCTATAGAACAGACAGTCAAGAAAGATGTCAAGTGTGACCTGCCTGGTCAAGGCTTCCT
           P I E Q T V K K D V S V T C L V T G F L
1261    CCCTCAGGACATCCACGTGGAATGGGAGAGCAATGGGCAGCCACAGCCAGAGCAGAACTA
           P Q D I H V E W E S N G Q P Q P E Q N Y
1321    CAAGAACACCCAGCCTGTCTTGGACTCCGATGGCTCTTACTTCCTGTACAGCAAGCTCAA
           K N T Q P V L D S D G S Y F L Y S K L N
1381    TGTGCCCAAGAGCAGGTGGGACCAGGGAGATTCCCTTCACCTGCTCCGTGATACATGAGGC
           V P K S R W D Q G D S F T C S V I H E A
1441    TCTGCACAACCACCATGACGAAGACCATCTCCCGGTCTCTGGGTAATTGAGCTCAGCA
           L H N H H M T K T I S R S L G N *
1501    CCCAGAAAGCTCTTAGGTCTAAGCTACCCCGGCACCTCCTCCACCTTCCCTTGTATA
1561    AATAAAGCACCCAGCACTGCCCTGGG

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Fig. 1.
Nucleotide sequence and deduced translation of HM79-16 heavy chain. The predicted leader region cleavage site is indicated by “//”. The polyadenylation signal is underlined. Conserved amino acid cysteine 23 (1st-CYS) and cysteine 104 (2nd-CYS) are highlighted (Lefranc, 2011b; Lefranc et al., 2003; Lefranc et al., 2005). The full-length sequences have been deposited in GenBank (accession number KC294425).


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1   GTAATGAAACAGCCCCAACAGAAGATTCTGGTCTAAGAAGAAGCTGTGTTGTGCCTAAAA

61  TGGCTTGGACTCCTGGCATCTTCATGGTTCTGTCTTACTTGACAGGTTCTTTTCTCAGT
    M A W T P G I F M V L S Y L T G S F S //Q
121 CTATATTGACACAACCACCTCCATCTCTGAGTCTCTTGGATCAACAGCCAGACTCACCT
    S I L T Q P P S I S E S L G S T A R L T
181 GCACCCTGAGGAGTGGCATCAGTGTGGTAATAAAATATTTACTGGTACCAGCAAATGG
    C T L R S G I S V G N K N I Y W Y Q Q M
241 CGGGGAGTGTCTCCTCGTTTGTTCCTGTACTTCTTCTCAAATTCAGACAAGGAGCTGGGGC
    A G S A P R L F L Y F F S N S D K E L G
301 CTGGAGTCCCCAACAGAGTCTCTGGATCCAAAGATACCTCCAAAAATGCTGCAAATTTGC
    P G V P N R V S G S K D T S K N A A N L
361 AGATCTCTGACCTACAGGTGGAGGATGAGGCTGTGTATTACTGTCCATCTATGAAAGTA
    Q I S D L Q V E D E A V Y Y C S I Y E S
421 ATGCGTATGTTTTTGGCAGCGGAACCCAGCTCACCGTCTAGGTGGACCAAGTCTTCTC
    N A Y V F G S G T Q L T V L G G P K S S
481 CCAAAGTCACAGTGTTCACCTTCACCTGAGGAGCTCCGGACAAACAAAGCCACACTGG
    P K V T V F P P S P E E L R T N K A T L
541 TGTGTCTGGTTAATGACTTCTACCCGGGTTCTGCAACAGTGACCTGGAAGGCAAATGGAG
    V C L V N D F Y P G S A T V T W K A N G
601 CAACTATCAATGATGGGGTGAAGACTACAAAGCCTTCCAAACAGGGCCAAAACATACATGA
    A T I N D G V K T T K P S K Q G Q N Y M
661 CCAGCAGCTACCTAAGTTTGACAGCAGACCAGTGGAATCTCACAACAGGGTTTCCTGCC
    T S S Y L S L T A D Q W K S H N R V S C
721 AAGTTACCCATGAAGGGGAAACTGTGGAGAAGAGTTGTCCCCTGCAGAATGTCTCTAGG
    Q V T H E G E T V E K S L S P A E C L *
781 AGCCCAGTCTTTTCTTAGCCCAGGAAGCCTGGAGCTACGGGACCAGAATGTGGTCTTC

841 TCTCTATTCTATCAATCTCAACCTTCTCTTACCCACTGAGTATTCAATAAAGTATCA

901 TTAGTTAATCAG

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Fig. 2.

Nucleotide sequence and deduced translation of HM79-16 light chain. The predicted leader region cleavage site is indicated by “//”. The polyadenylation signal is underlined. Conserved amino acid cysteine 23 (1st-CYS) and cysteine 104 (2nd-CYS) are highlighted (Lefranc, 2011b; Lefranc et al., 2003; Lefranc et al., 2005). The full-length sequences have been deposited in GenBank (accession number KC294424).

Fig. 3. IMGT protein displays for V domains of HM79-16 and HL4E10. VH and V-LAMBDA sequences of HM79-16 (KC294425 and KC294424) and HL4E10 (HM369133 and HM369134) are aligned using IMGT/DomainGapAlign (Ehrenmann and Lefranc, 2011).

Fig. 4.

IMGT protein displays for C regions of HM79-16 and HL4E10. The heavy gamma C region comprises three domains (CH1, CH2, CH3), the hinge between CH1 and CH2, and the CHS (present at the C terminal end of a secreted chain). CH1, hinge, CH2, CH3, CHS of HM79-16 and HL4E10 are presented successively (top). The light lambda C region comprises a single C domain (C-LAMBDA). C-LAMBDA of HM79-16 (KC294424), HL4E10 (HM369134), and mouse IGLC1 (J00587), IGLC2 (J00595), IGLC3 (J00585) are shown (bottom). Sequences are aligned using IMGT/DomainGapAlign (Ehrenmann and Lefranc, 2011).

Fig. 5. IMGT Collier de Perles of the C-LAMDA for HM79-16 (A) and HL4E10 (B). IMGT Colliers de Perles were obtained using the IMGT/Collier-de-Perles tool (Ehrenmann et al., 2011), accessed from IMGT/DomainGapAlign.

Fig. 6.

Phylogenetic tree of the IGLC regions of different species. The tree was generated by the neighbor-joining method using ClustalW2 program (Larkin et al., 2007). The bar (0.1) indicates the genetic distance. The IGLC regions of various species were obtained from IMGT®, the international ImMunoGeneTics information system® (<http://www.imgt.org>), IMGT/GENE-DB (Giudicelli et al., 2005; Lefranc et al., 2009). The name and accession numbers (in parentheses) are: human IGLC1 (J00252), IGLC2 (X06875), IGLC3 (J00254), IGLC6 (J03011), IGLC7 (X51755); mouse IGLC1 (J00587), IGLC2 (J00595), IGLC3 (J00585); rabbit IGLC1 (M12388), IGLC2 (M12761), IGLC3 (M12762), IGLC4 (M12763), IGLC5 (M23227), IGLC6 (M23229); Rat IGLC1 to 4 (AC111360). The standardized IMGT 6-letter abbreviations are used: Homsap (*Homo sapiens*, human), Musmus (*Mus musculus*, house mouse), Orycun (*Oryctolagus cuniculus*, rabbit), Ratnor (*Rattus norvegicus*, Norway rat), Crimig (*Cricetulus migratorius*, Armenian hamster).

Table 1

DNA sequence percent of identity of the IGLC regions

	Hamster			Mouse		
	HM79-16	HL4E10	H28.710	IGLC1	IGLC2	IGLC3
HM79-16		74.5	48.0	72.7	80.1	80.4
HL4E10			37.3	80.5	75.2	76.1
H28.710				45.4	35.6	46.3
IGLC1					73.2	84.4
IGLC2						96.4
IGLC3						44.7

DNA sequences of the IGLC regions of three hamster hybridomas (HM79-16, HL4E10, H28.710) and four mouse genes (IGLC1, IGLC2, IGLC3, IGKC) were compared in a pairwise manner. Each number in the table shows the percentage of identity between the nt sequences of the corresponding row and column.