Journal of Immunological Methods 405 (2014) 35–46

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Analysis of heavy and light chain sequences of conventional camelid antibodies from *Camelus dromedarius* and *Camelus bactrianus* species ☆

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ARTICLE INFO

Article history:
Received 11 October 2013
Received in revised form 23 December 2013
Accepted 7 January 2014
Available online 18 January 2014

Keywords:
Camel IgG1
CDR length
CH1 domain
Kappa
Lambda
VHH

ABSTRACT

Camel antibodies have been widely investigated, but work has focused upon the unique heavy chain antibodies found across camelid species. These are homodimers, devoid of light chains and the first constant heavy chain domain. Camelid species also display conventional hetero-tetrameric antibodies with identical pairs of heavy and light chains; in *Camelus dromedarius* these constitute 25% of circulating antibodies. Few investigations have been made on this subset of antibodies and complete conventional camel IgG sequences have not been reported.

Here we study the sequence diversity of functional variable and constant regions observed in 57 conventional heavy, 18 kappa and 35 lambda light chains of *C. dromedarius* and *Camelus bactrianus*. We detail sequences of the full kappa and lambda light chain, variable and CH1 region for IgG1a and IgG1b and the CH2 and CH3 region for IgG1a. The majority (60%) of IgG1 variable region sequences aligned with the human IgHV3 family (clan III) and had leader sequences beginning with MELG whereas the remaining sequences aligned with the IgHV4 (clan II) and had leader sequences beginning with MRLL. Distinct differences in CDR length were observed between the two; where CDR1 was typically 5 and 7 residues and CDR2 at 17 and 16 residues, respectively. CDR3 length of IgHV4 (range 11 to 20) was closer to that typical of VHH antibodies than that of IgHV3 (range 3 to 18 residues). Designed oligonucleotide primers have enabled identification of paired heavy and light chains of conventional camel antibodies from individual B cell clones.

Abbreviations: C1q, multivalent complex and immunoglobulin binding component of the complement activation cascade; CDR, complementarity determining region; CH1 CH2 & CH3, first, second and third heavy chain constant region domains; FcRn, neonatal Fc receptor; Cκ, kappa light chain constant region; Cλ, lambda light chain constant region; FCS, fetal calf serum; HCAbs, heavy chain antibodies; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IgG1a IgG1b, IgG2a, IgG2c, IgG3, immunoglobulin gamma isotypes; IgHV3 and IgHV4, immunoglobulin heavy chain variable region sub-groups; MELG and MRLL, single letter amino acid code; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RPMI media, Roswell Park Memorial Institute media; VH, variable region of conventional antibodies; V-regions, variable region; V–D–J, variable diversity and joining region genes; VHH, variable domain of heavy chain antibody.

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http://dx.doi.org/10.1016/j.jim.2014.01.003

1. Introduction

The hetero-tetrameric heavy and light chain IgG antibody format formed the central dogma for antibody structure until 1993 when a novel antibody structure was discovered in the camelid species *Camelus dromedarius* (Hamers-Casterman et al., 1993). These novel antibodies, termed heavy chain antibodies (HCAbs), were shown to be heavy chain homodimers, which did not associate with a light chain, and lacked the CH1 domain (Muyldermans et al., 1994; Nguyen et al., 1999). Immunological investigation of all members of the *Camelidae* family has shown the co-existence of conventional and heavy chain antibodies (Hamers-Casterman et al., 1993; Maass et al., 2007; Van der Linden et al., 2000). In camels, heavy chain antibodies constitute 75% of circulating antibodies (Hamers-Casterman et al., 1993). To distinguish them from conventional antibody heavy chain variable regions (VH), the variable domain of heavy chain antibodies has been denoted VHH. The unique structure and novel characteristics of this new class of antibodies are of commercial significance with VHH fragments (known as nanobodies) forming a new class of therapeutics used to treat human diseases (Muyldermans et al., 2009). The extended CDR-H3 region characteristic of VHH fragments, and their preference for binding protein clefts, makes them an interesting tool in the pharmaceutical industry (Lawson, 2012).

Since their discovery, considerable research has been undertaken to understand the evolution, sequence diversity and biophysical characteristics of the novel heavy chain antibodies. A wealth of sequence information is available for this novel subset of antibodies, however considerably fewer investigations have been made into the sequence diversity of conventional camel antibodies. Comparisons have been made of VHH and VH region sequences (Vu et al., 1997; Harmsen et al., 2000; Nguyen et al., 1998; Nguyen et al., 2000) and numerous publications document camel conventional antibody hinge sequences. However, assessment of the full constant region sequences for IgG1a and IgG1b is lacking, with only a single publication providing nucleotide sequence information for an IgG1 constant region, from a non-rearranged llama genomic DNA library (Woolven et al., 1999). In addition to a paucity of conventional heavy chain data, a complete lack of light chain data exists. Although reference has been made to camel light chain sequence investigations (Legssyer et al., 1995; De Genst et al., 2006), no publication of the data has been identified. The complete sequencing of the *C. dromedarius* genome is underway (Al-Swailem et al., 2010) and inspection of deposited expressed sequence tag data indicates that there are 5 lambda constant region genes and only one kappa constant region gene (http://camel.kacst.edu.sa/). Similarly a draft genome of wild and domesticated Bactrian camels has recently been reported (Jirimutu et al., 2012).

The existence of two structurally distinct types of antibody variable regions in camelids offers the opportunity to compare structure and functional relationships of VHH and conventional combining sites resulting from an immune response to a defined antigen. As a necessary prelude to such a study, here we characterize heavy and light chain sequence diversity of dromedary and Bactrian IgG1utilizing RACE technology. This has allowed design of oligonucleotide primers that enable the identification of paired heavy and light chain sequences from individual camel B cells.

2. Materials and methods

2.1. Source material

PBMCs were sourced from ten dromedary and two Bactrian out-bred camels maintained at the Central Veterinary Research Laboratory (CVRL) Dubai. The camels had not been purposely immunized for this study. Blood sampling was performed according to a humane protocol approved by the Scientific Directorate of the CVRL.

2.2. PBMC mRNA extraction

To remove non-viable cells, PBMC were cultured overnight in RPMI media (10% FCS, 2 mM glutamine, 20 mM HEPES, 55 nM 2-mercaptoethanol, 100 U/ml penicillin, 100 ug/ml streptomycin). RNA was extracted from approximately 5×10^6 PBMC from individual camels using Qiagen RNEasy kits, according to the manufacturer's instructions. RNA was stored at −80 °C.

2.3. Heavy and light chain cDNA preparation

RACE-ready cDNA templates, both 5′ and 3′, were prepared for each RNA sample, as detailed in the SMART RACE protocol (BD Biosciences). Dromedary and Bactrian pools of cDNA were prepared, with the amount of cDNA added to each pool standardized according to the initial cell number from which the RNA was derived.

Antisense oligonucleotides CALL002 (GGTACGTGCTGTTGA ACTGTTCC) (Conrath et al., 2001) and CH2FORTA4 (CGCCATC AAGGTACCAGTTGA) (Ghahroudi et al., 1997), which annealed in the CH2 region, were employed in the heavy chain 5′ RACE PCR reaction. To assess sequence variability in the heavy chain Fc region a heavy chain 3′ RACE PCR reaction was undertaken utilizing individual hinge region specific sense oligonucleotides (1a: CAACCACAACCAGGATGCACG, 1b: GAACCGCATGGAGGATG CCCG). These oligonucleotides were designed for each isotype based upon sequence information gained in the heavy chain 5′ RACE experiment.

Due to the absence of published camel light chain sequences, an initial kappa light chain 5′ RACE PCR reaction was conducted using a non-camel specific oligonucleotide (CTGCTCAVTGGAT GGTGGGAAGA) designed based upon an alignment of homologous regions of the kappa light chain constant domains of human, rabbit and mouse sequences. Sequence analysis of the resultant PCR product isolated two camel kappa light chain sequences. These sequences were used to re-design a camel-specific framework 3 oligonucleotide (GCTGATCGTGAGAGTGA AATC, antisense) and a leader sequence specific oligonucleotide (ATGGGGCCACAGCCACGCCTGCTCAC, sense) for use in the 5′ and 3′ camel kappa light chain RACE experiments respectively. Lambda light chain 3′ RACE investigations were undertaken using a documented sense oligonucleotide (GCCGGCCATGGCCT GGGCTCTGCTCC) (Conrath et al., 2005).

All RACE PCR reactions were undertaken as defined in the SMART RACE protocol, using oligonucleotides detailed above and KOD hot start polymerase. RACE PCR products were analyzed by gel electrophoresis. 5′ heavy chain RACE PCR products were observed to migrate in a triple banding pattern. The largest band was predicted to represent conventional heavy chain antibodies (IgG1a and IgG1b); the two smaller bands