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Exceptionally long CDR3H of bovine scFv antigenized with BoHV-1 B-epitope generates specific immune response against the targeted epitope

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ABSTRACT

We discovered that some bovine antibodies are amongst the largest known to exist due to the presence of an exceptionally long CDR3H (≥49 amino acids) with multiple cysteines that provide a unique knob and stalk structure to the antigen binding site. The large CDR3H size, unlike mouse and human, provides a suitable platform for antigenization with large configurational B-epitopes. Here we report the identification of a B-epitope on the gC envelope protein of bovine herpes virus type-1 (BoHV-1) recognized by a bovine IgG1 antibody. The identified 156 amino acid long gC fragment (gC156) was expressed as a recombinant protein. Subsequently, a functional scFv fragment with a 61 amino-acid long CDR3H (scFv1H12) was expressed such that gC156 was grafted into the CDR3H, replacing the "knob" region (gC156scFv1H12 or Ag-scFv). Importantly, the Ag-scFv could be recognized by a neutralizing antibody fragment (scFv3-18L), which suggests that the engraftment of gC156 into the CDR3H of 1H12 maintained the native conformation of the BoHV-1 B-epitope. A 3D model of gC156 was generated using fold-recognition approaches and this was grafted onto the CDR3H stalk of the 1H12 Fab crystal structure to predict the 3D structure of the Ag-scFv. The grafted antigen in Ag-scFv is predicted to have a compact conformation with the ability to protrude into the solvent. Upon immunization of bovine calves, the antigenized scFv (gC156scFv1H12) induced a higher antibody response as compared to free recombinant gC156. These observations suggest that antigenization of bovine scFv with an exceptionally long CDR3H provides a novel approach to developing the next generation of vaccines against infectious agents that require induction of protective humoral immunity.

1. Introduction

Antibody antigenization (Zanetti et al., 1992) is based on the concept of idiotypic networks where internal images of natural antigens are genetically programmed during B- and T-cell development (Jerne, 1974). Apart from immunoregulatory functions of the idiotypic network, it provides a theoretical basis for mimicking a natural antigen via T- and B-epitope grafting into the complementarity determining regions (CDRs) to induce a desired immune response. Thus, the CDRs supported by the framework-regions are capable of providing the molecular environment to express epitopes in a configurationally suitable and immunologically accessible manner. In this context, murine immunoglobulin (Ig) antigenized with T- and B-epitopes from viral (Brumeanu et al., 1996), protozoan (Billetta et al., 1991) and other antigens, such as, CD4 (Lanza et al., 1993) have been shown to elicit the desired immune responses. The antigenization of mouse or human CDRs is constrained due to their relatively short size and does not permit expression of larger discontinuous or configurational B cell epitopes.

We earlier discovered that some bovine antibodies are among the largest known to exist because of an exceptionally long CDR3 of the heavy chain (CDR3H; ≥49 codons) containing multiple cysteine residues (Kaushik et al., 2002, 2009; Saini et al., 1999; Saini and Kaushik, 2002). These bovine antibodies with an atypical CDR3H

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Table 1
Primers used to construct various recombinant proteins.

No.	Primer	Nucleotide Sequence	Size (bp)
1[a]	s-sFvVJ1H12	GTGGCCCAGCCGGCCCAGGCTGTGCTGAATCAG	33
2	as-FvVJ1H12-L	GGAAGATCTAGAGGACTGACCCAGGACGGTCAGTGTGGT	39
3	s-FvVDJ1H12-L	GGTCAGTCCTCTAGATCTTCCGGCGGTGGTGGCAGCTCCGGTGGTGGCGGTTCCCAGGTGCAGCTGCG	68
4[a]	as-FvVDJ1H12	CTGGCCGGCTTGGCCACTAGTTGAGGAGACGGTGACCAG	39
5	as-FvVJVD1H12-L	GTAAGATCCGCCACCACCCGAACGTTCTCTATAC	34
6	s-DJ1H12-L	GTACGGAGGAGGAGGCAGTTATAGTTATACTTAC	34
7	s-gC156	GTGGCCCAGCCGGCCTACCCCGTGGAGG	28
8	as-gC156	CTGGCCGGCTTGGCCGTACGTGGCGGTCGC	30
9	s-gC156-L	GGTGGTGGCGGATCTTACCCCGTGGAGGCTC	31
10	as-gC156-L	CTGCCTCCTCCTCCGTACGTGGCGGTCGCG	30

[a] Used to construct scFv1H12 and gC156scFv1H12; s—sense, as—antisense primers. Primers 1–4 were used to construct scFv1H12 from BLV1H12; Primers 7 and 8 were used to amplify gC156 for recombinant protein expression.

are present across all antibody classes (Walther et al., 2013) and show restricted $V_H + V_L$ pairing (Saini et al., 2003). These antibodies are encoded by restricted *VDJ* recombinations (Aida et al., 2015; Pasman and Kaushik, 2014) utilizing single long *IGDH(DH2)* (Shojaei et al., 2003) and involve insertion of A and T nucleotide rich 'CSNS' specifically at the V_H-D_H junction (Koti et al., 2010b). Such atypical bovine CDR3H display the structural properties that should facilitate the incorporation of a relatively large conformational B-epitope. Recently, the crystal structure was determined for a bovine Fab (BLV1H12) with a 61 residue long CDR3H, which adopted an elongated "stalk-knob" structure (Wang et al., 2013). The stalk protruded as an extended pair of β-strands typical of long CDR3H regions (Ramsland et al., 2001). However, the cysteine-rich region formed a globular head domain termed the "knob", which was highly accessible and spatially separated from the bulk of the variable domains. For these reasons, bovine antibody or its fragments provide a suitable platform for antigenization, particularly in the CDR3H, with single or multiple discontinuous B-epitopes together with appropriate T-epitopes to induce adaptive immune responses. Antigenization involves use of an Ig variable-region scaffold to present epitope(s) that are correctly folded and accessible for antibody recognition, two features important for induction of an effective immune response. Compared to earlier studies that antigenized whole Ig, we now extend the concept to antigenizing only variable (V) regions by expressing single chain variable fragments (scFv), consisting of the heavy and light chain V domains joined by a flexible linker.

Bovine herpes virus-1 (BoHV-1) is an important cattle pathogen involved in bovine respiratory disease complex (Muylkens et al., 2007). Vaccination against BoHV-1 does not prevent infection as BoHV-1 establishes latency resulting in its reactivation during stress. Thus, there is a need to develop better vaccines and therapeutic anti-viral drugs to control BoHV-1 infection. In this context, we earlier developed potent bovine scFvs capable of neutralizing BoHV-1 (Koti et al., 2010a; Koti et al., 2011; Pasman et al., 2012) for passive immunization.

In this study, we have identified a 32-kDa B cell epitope present on the gC envelope glycoprotein of BoHV-1 recognized by neutralizing scFvs (Koti et al., 2010a; Koti et al., 2011; Pasman et al., 2012). We then expressed the 156 amino acid gC sub-fragment (17 KDa) in *P. pastoris*, predicted to contain linear T cell epitopes and a large conformational (or configurational) B cell epitope. Further, we constructed functional scFv (scFv1H12) with an exceptionally long CDR3H (61 amino acids) (Pasman and Kaushik, 2016) where the 17 kDa gC sub-fragment (gC156) was grafted into the CDR3H (gC156scFv1H12; referred to as Ag-scFv). Importantly, both gC156 and Ag-scFv were recognized by the BoHV-1 neutralizing scFv indicating conformational stability of the target epitopes in free and antigenized form. Recombinant gC156 and Ag-scFv were used to immunize bovine calves to assess generation of targeted immune response. While the recombinant gC156 induced a specific antibody response, the antigenized scFv produced higher antibody titers as compared to free gC156. Thus, antigenization of bovine scFv with an exceptionally long CDR3H provides a novel approach to developing new vaccines against infectious agents.

2. Materials and methods

2.1. Identification of the B-epitope recognized by BoHV-1 neutralizing scFv

Since anti-BoHV-1 scFv3-18L (Koti et al., 2011) recognizes a B-epitope on a 32 kDa protein in the envelope fraction of BoHV-1, the envelope fraction was prepared from 500 μg of purified virus and gel fractionated using 12% SDS-PAGE. The 32 kDa protein band was subjected to in-gel trypsin digestion followed by mass spectrometry (McMaster Regional Centre for Mass spectrometry, Hamilton, Canada). The identity of the protein sequence was determined using Mascot™ software (Perkins et al., 1999). The theoretical secondary structure of a 17 kDa gC subfragment (gC156) containing the identified peptide sequence was visualized in Geneious version 6.1.8 (Kearse et al., 2012) that predicted antigenic regions (EMBOSS™ Antigenic) and cleavage sites (EMBOSS™ Garnier) (Rice et al., 2000).

2.2. Construction of recombinant gC subfragment (gC156)

BoHV-1, Cooper strain, gC envelope glycoprotein fragment (UL44; GenBank NC_001847 16,818–17,285; 468 nucleotide sequence encoding 156 amino acids), designated as gC156, was PCR amplified from BoHV-1 using sense and anti-sense primers with built-in *SfiI* restriction sites (Table 1, primers 7 and 8), followed by cloning into pPICZα vector (Invitrogen USA) and expression in *P. pastoris,* as described (Koti et al., 2010a; Koti et al., 2011; Pasman et al., 2012).

2.3. Construction of antigenized scFv with putative BoHV-1 B-epitope

The scFv with exceptionally long CDR3H (scFv1H12-18L) was first constructed (Pasman and Kaushik, 2016) from RNA isolated from BLV1H12 hybridoma (Saini et al., 1999; Saini et al., 1997). The scFv antigenization strategy involved grafting of gC156 (468 nucleotide sequence encoding 156 amino acids) with flanking five amino acid linkers (Gly_4Ser) into the exceptionally long CDR3H of scFv1H12. Briefly, construction of the Ag-scFv (gC156scFv1H12) involved sequential overlapping PCR amplifications (Table 1 primer 1–4, 9 and 10) under following conditions: denaturation 98 °C 30 s followed by thirty amplification cycles (98 °C 15s, annealing 61 °C 20 s, extension 72 °C 30 s) and a final extension step at 72 °C for 7–15 min (iProof BioRad, USA). Similar PCR conditions were used for incorporating the flanking linkers with the gC156 with the