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Research paper

Somatic hypermutations and isotype restricted exceptionally long CDR3H contribute to antibody diversification in cattle

Azad K. Kaushik [a,*], Marcus E. Kehrli Jr. [b], A. Kurtz [a], S. Ng [a], M. Koti [a], F. Shojaei [a], Surinder S. Saini [a]

[a] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[b] Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center-USDA-Agricultural Research Service, 2300 Dayton Avenue, Ames, IA 50010, USA

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ABSTRACT

Antibody diversification in IgM and IgG antibodies was analyzed in an 18-month old bovine (*Bos taurus*) suffering from naturally occurring chronic and recurrent infections due to bovine leukocyte adhesion deficiency (BLAD). The BLAD, involving impaired leukocyte β2 integrin expression on leukocytes, develops due to a single point mutation in conserved region of the CD18 gene resulting in substitution of aspartic acid128 with glycine (D128G). Twenty four VDJCμ and 25 VDJCγ recombinations from randomly constructed cDNA libraries, originating from peripheral blood lymphocytes, were examined for the variable-region structural characteristics in IgM and IgG antibody isotypes. These analyses led to conclude that: (a) expression of exceptionally long CDR3H is isotype restricted to cattle IgM antibody; (b) VDJ recombinations encoding IgM with exceptionally long CDR3H undergo clonal selection and affinity maturation via somatic mutations similar to conventional antibodies; (c) somatic mutations contribute significantly to both IgM and IgG antibody diversification but significant differences exist in the patterns of 'hot spot' in the FR1, FR3 and CDR1H and, also, position-dependant amino acid diversity; and (d) transition nucleotide substitutions predominate over transversions in both VDJCμ and VDJCγ recombinations consistent with the evolutionary conservation of somatic mutation machinery. Overall, these studies suggest that both somatic mutations and exceptional CDR3H size generation contribute to IgM and IgG antibody diversification in cattle during the development of immune response to naturally occurring chronic and multiple microbial infections.

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1. Introduction

The immunoglobulins (Ig) are B cell antigen receptors (BCR) and constitute the effectors of adaptive humoral host defences, including those against infectious agents, for which generation of extensive receptor diversity is essential. However, variable-region diversification, both for heavy- (H) and light- (L) chain of an antibody, varies significantly across jawed vertebrates (Butler, 1998; Davis, 2004; Maizels, 2005; Market and Papavasiliou, 2003; Saini and Kaushik, 2003) depending upon relative contribution of germline based combinatorial diversity and post-recombinational mechanisms, e.g., somatic hypermutations and gene conversion. The relative role of H- and L-chains in antigen recognition also varies considerably depending upon antigen specificity or the species involved.

In mice and humans, diverse variable (V) germline genes in juxtaposition with diversity (D) and joining (J)

* Corresponding author at: Department of Molecular and Cellular Biology, Room SCIE 4248, University of Guelph, Guelph, Ontario N1G 2W1, Canada Tel.: +1 519 824 4120.
E-mail address: akaushik@uoguelph.ca (A.K. Kaushik).

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genes produce significant diversity, via combinatorial V(D)J recombinations, of the variable-region of heavy chains in the bone marrow throughout life (Kirkham and Schroeder, 1994; Schatz, 2004). Further, most diversity resulting from the amino acid composition is concentrated in the third complementarity-determining region (CDR) of the heavy-chain (CDR3H) that is located in the center of the epitope-binding site. The species with limited germline diversity at the variable-region locus of both the heavy- (V_H) and light- ($V_{\kappa/\lambda}$) chains, such as, chicken (Reynaud et al., 1987), rabbit (Mage, 1998), swine (Butler and Wertz, 2006), sheep (Reynaud et al., 1995) and cattle (Kaushik et al., 2002; Zhao et al., 2006), utilize different post-recombination diversification strategies of the variable region of the antibody heavy-chain since it is mainly involved in antigen recognition. While the single functional V_H and V_λ gene present in chickens is diversified via gene conversion in the bursa of Fabricius (Reynaud et al., 1987), both gene conversion and somatic hypermutations in the appendix contribute to the diversification process in rabbits (Mage, 1998). Select members of gut microflora are also suggested to drive the gut-associated lymphoid tissue (GALT) development in rabbits (Lanning et al., 2000). In swine, extensive junctional diversity provides the major source of diversification during preimmune repertoire development (Butler and Wertz, 2006). Yet a non-antigen dependent somatic hypermutation process during pre-immune repertoire development generates antibody diversity in sheep (Reynaud et al., 1995). A single polymorphic V_H gene family, $BOVV_H1$, is identified in cattle (*Bos taurus*) that is predominantly expressed in the primary antibody repertoire (Berens et al., 1997; Saini et al., 1997; Sinclair et al., 1997). Three V_λ gene families, $V_\lambda1$, $V_\lambda2$ and $V_\lambda3$, are known in cattle (Saini et al., 2003a) where $V_\lambda1a$ and $V_\lambda1b$ genes are mostly expressed in bovine antibodies (Parng et al., 1996; Sinclair et al., 1995). Interestingly, the antibody diversity in cattle also originates from the generation of exceptionally long CDR3H regions (Saini et al., 1999). These unusually long CDR3H regions can extend up to 61 residues in size and often contain multiple even numbered Cys residues. Although multiple Cys residues have been observed in antibody CDR3H of other vertebrates like sharks, camels, dolphins, monotremes (platypus), and occasionally in humans, these Cys-rich CDR3H do not attain the massive size seen in cattle antibodies (Ramsland et al., 2001). The exceptionally long CDR3H regions in cattle antibodies may originate from those germline D_H genes that are unusually long as compared to other species (Shojaei et al., 2003). A unique characteristic of bovine antibodies with exceptionally long CDR3H is that these specifically pair with $V_\lambda1x$, $V_\lambda1d$ and $V_\lambda1e$ genes (Saini et al., 2003a) with conserved Ser90 and Ala91 in the CDR3L regions. The role of the λ-light chains in cattle antibodies is essentially to act as a supporting platform for the extremely long CDR3H regions which are dominantly involved in binding to an antigen (Saini et al., 2003a).

Bovine leukocyte adhesion deficiency (BLAD) is an autosomal recessive genetic disease (Gerardi, 1996; Kehrli et al., 1992; Nagahata, 2004) of cattle resulting from impaired β2 integrins expression on leukocytes due to a single point mutation in conserved region of the CD18 gene resulting in substitution of aspartic acid128 with glycine (D128G). An additional silent mutation involving replacement of cytosine to thymine at position 775 of CD18 gene is also noted (Shuster et al., 1992). The integrins are heterodimeric proteins consisting of various α chains (CD11a, b, c) non-covalently pairing with the common β chain (CD18) that function as adhesion molecules. Thus, the primary immunodeficiency is characterized with peripheral lymphadenopathy, neutrophilia, hypoalbunemia and hypergammaglobulinemia. The inhibition of diapedesis in the inflammatory response prevents development of normal immune responses to invading pathogens leading to chronic and recurrent infections in BLAD afflicted cattle. The antigen-specific immune responses to tetanus toxoid and rabies virus in BLAD cattle are known to be delayed and impaired (Nagahata et al., 1994). For these reasons, BLAD afflicted cattle provide an immunodeficient model to examine the degree of somatic diversification in IgM and IgG antibody isotypes. The experiments outlined here involved structural analysis of IgM and IgG antibodies from a single BLAD afflicted cattle where these arose as a result of selective pressures on the responding antibody repertoire due to natural infections. These studies demonstrate that cattle IgM and IgG antibodies bear distinct structural properties with regard to exceptional CDR3H size and patterns of somatic hypermutations in the variable-regions during the development of immune response to natural chronic infections.

2. Materials and methods

2.1. Animal

An approximately 18-month-old Holstein heifer afflicted with BLAD was used to obtain lymphocytes. This calf was typical in its clinical presentation (recurrent respiratory disease and diarrhea, a persistent progressive neutrophilia, stunted growth, periodontitis) and was homozygous for the D128G CD18 allele. Peripheral blood was collected into 2× acid citrate dextrose (10%, v/v) by jugular venipuncture. Anticoagulated blood was centrifuged and the plasma layer was discarded. Mononuclear cells from blood were enriched from whole blood buffy coats by buoyant density centrifugation for 40 min at 450 × *g* over a colloidal polyvinylpyrolidone-coated silica gradient (sp gr 1.084). Lymphocyte-enriched cells were harvested and washed once in physiological saline solution and resuspended in Trizol (Gibco BRL, Gaithersburg, MD). The mRNA obtained from lymphocytes was used for construction of two cDNA libraries.

2.2. Polymerase chain reaction (PCR)

Single strand cDNA was synthesized from 7 μg of total RNA, isolated by Trizol reagent (Gibco-BRL, Burlington, Ontario), using polydT primer (Amersham Pharmacia Biotech, Baie d'Urfé, Quebec). All VDJ recombinations were PCR amplified (Saini et al., 1997) in an unbiased manner using high fidelity HF Expand system (Boehringer-