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Antibody repertoire development in the sheep

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Abstract

The model of immunoglobulin (Ig) repertoire diversification in sheep has evolved dramatically in recent years. A process thought to involve the rearrangement of a very limited number of variable (V), diversity (D) and joining (J) segments followed by intense, antigen (Ag)-independent, somatic hypermutation is now known to be less recombinatorially restrictive and to involve fewer mutational events. Although mutation rates are now lower than previously thought, the somatic hypermutation process itself is no less critical to the development of the primary Ig repertoire in sheep. Recent studies have shown that those B cells that fail to mutate will die via apoptosis. Much of the V(D)J rearrangement is thought to occur in the fetal liver and spleen prior to development of the ileal Peyer's patch (PP) at approximately day 100 of gestation. Although de novo Ig rearrangement likely does not occur in the ileal PP, this tissue is a site of massive B-cell proliferation, selection and Ig diversification through somatic hypermutation.

Keywords: B lymphocytes; Immunoglobulins; Repertoire development; Germline repertoire; Gene rearrangement; Junctional diversity; Somatic hypermutation; Ileal Peyer's patch

1. The sheep germline repertoire

1.1. Variable (V) gene segments

Our current understanding of the sheep germline Ig repertoire is limited but it is slowly being resolved as more and more data is generated. It was believed from the first studies on sheep Ig repertoires that the germline repertoires of the Ig H and L chains were very restricted [1–3], however, emerging evidence from larger scale molecular studies suggests otherwise [4,5].

Initial studies on the sheep Ig H chain locus had identified three VH gene segments and probing of a genomic DNA library and further PCR based analysis

Abbreviations Ag, antigen; C, immunoglobulin constant gene segment; CDR, complementarity determining region; D, immunoglobulin diversity gene segment; FS del, frame-shift deletion; FR, framework region; GALT, gut-associated lymphoid tissue; H, immunoglobulin heavy chain; Ig, immunoglobulin; J, immunoglobulin joining gene segment; L, immunoglobulin light chain; LPS, lipopolysaccharide; N, non-templated nucleotide; nt, nucleotide; P, palindromic nucleotide; PBL, peripheral blood lymphocytes; PP, Peyer's patch; RACE, rapid amplification of cDNA ends; RSS, recombination signal sequence; R, replacement mutation; S, silent mutation; TLR, Toll-like receptor; V, immunoglobulin variable gene segment.

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Table 1a
Ovine VH nomenclature

Description	H Family	GenBank accession number	Other information
VG1U	1	Z49180	Derived from PCR
VGE7U	1	Z49181	
VGE6U	1	Z49182	
VG5U	1	Z49183	Derived from PCR. Likely pseudogenes (11 nt deletion in leader sequence). VG5U also lacks the ATG START codon
VG2U	1	Z49184	
VG10U	1	Z49185	
V7A	1	Z49186	Obtained from genomic library
V5A	1	Z49187	
V1B	1	Z49188	

had identified an additional six VH gene segments for a total of nine sheep VH gene segments (Table 1a) [3]. Together these data correlated well with locus size predictions made from Southern blotting using two sheep VH probes and probes corresponding to six of the seven human VH families [3]. Sequence analysis of these clones indicated that they belonged to a single gene family, designated V_H1, and shared the greatest similarity to either the human family V_H4 or to the mouse family V_H1 [3]. This single sheep VH family could be subdivided into two groups based on sequence patterns in the complementarity determining region (CDR) 2. Further analysis revealed that three of these germline VH gene segments appeared to be non-functional due to deletions within each leader sequence and they likely represent three pseudogenes within the sheep VH locus [3].

More recently, studies by other groups have identified a number of VH genes that do not match any of the nine published germline segments and this suggests the existence of a larger VH repertoire than previously reported. In one study a VH gene segment was identified with two additional codons located within the CDR1 [6]. Although it is possible that this sequence represents a novel germline gene segment, it is also possible that this sequence is derived from a post-rearrangement diversification event such as gene conversion.

In a second study, several VH gene sequences were identified that did not match any published sheep gene segments [4]. In fact, these sequences were so divergent that they did not even correspond to the single identified VH family but rather they indicated the presence of an additional eight VH families ($V_H2 \rightarrow V_H9$). These sequences were also amplified from rearranged gene segments and as such had likely undergone post-rearrangement diversification. However, they appeared to be so divergent from the previously published VH gene segments that it is unlikely they are simply a mutated form of a known germline VH gene. It is also possible that some of these new VH gene segments are divergent enough from the previously identified VH sequences that they would not have been detected by Southern blotting with sheep and human VH probes and as such would not have been included in the estimate of <10 germline VH gene segments [3]. Although it appears as though the sheep VH locus may contain more gene segments than the nine originally identified, the number remains relatively limited when compared with the number of gene segments in the VH loci of species such as humans and mice.

Less information is available on the germline genes of the Vκ locus. To date only six germline Vκ genes have been reported (Table 1b), and these correspond to four distinct Vκ families ($V_\kappa I \rightarrow V_\kappa IV$) [7]. Each of the four families appear to be used during early fetal development, however, by the later developmental stages genes from family III and IV are used more frequently in rearranged Ig molecules than are those of families I and II [7]. This limited number of Vκ genes likely does not restrict the final Ig repertoire in sheep to any great extent as only about 20–25% of all Ig molecules utilize the κ chain [8].

In many ways our understanding of the sheep Vλ locus parallels that of the VH locus. Early studies had

Table 1b
Ovine Vκ nomenclature

Description	κ Family	GenBank accession number	Other information
kappa-1	I	AF038133	Comparable use to kappa-3,4 in early fetal life but reduced rearrangement in later stages of development
kappa-2.1	II	AF038134	
kappa-2.2	II	AF038135	
kappa-2.3	II	AF038136	
kappa-3	III	AF038137	Dominate the rearranged Vκ pool in late fetal development
kappa-4	IV	AF038138	