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Developmental progression of immunoglobulin heavy chain diversity in sheep

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Abstract

In order to assess the respective impacts of combinatorial rearrangement, junctional diversification, somatic hypermutation and gene conversion in the generation of immunoglobulin heavy chain variable regions diversity, the sequences of 42 variable regions from late fetal, newborn and young sheep were determined and compared to those of adult animals. At earlier stages of development, the use of germline diversity segments appears restricted, junctional variability is already established, and somatic hypermutations are scarce. The sequence diversity in adults is much higher, which we suggest results from a higher hymermutation activity and possibly from the use of a variety of diversity segments. Altogether, this pattern is very reminiscent of the situation observed in cattle, except for the length of the third complementarity determining regions (CDR3) which are shorter in sheep than in bovine. Unlike the chicken and rabbit systems, it seems that new rearrangements continue to occur in sheep for at least several months after birth.

Keywords: Sheep; Development; Immunoglobulin heavy chain; Diversity; CDR3

1. Introduction

The establishment of the immunoglobulin light chain repertoire in the sheep has been studied extensively by several groups. B cell precursors are generated early in development, probably in fetal liver and bone marrow. They start colonizing the different gut-associated lymphoid tissues during the second half of gestation, and they are found in gut-associated follicles of the colon at day 60, in the jejunal Peyer's patches (JPP) around days 75–80, and finally in the

Abbreviations: CDR, complementarity determining region; CDR–IMGT, complementarity determining region as defined by IMGT; FR, framework region; FR–IMGT, framework region as defined by IMGT; HMS, hyper-mutated subset; *IGHD*, D heavy chain genes; *IGHJ*, J heavy chain genes; *IGHV*, V heavy chain genes; *IGKJ*, J kappa genes; *IGKV*, V kappa genes; *IGLJ*, J lambda genes; *IGLV*, V lambda genes; IMGT, International ImMunoGeneTics Information System®; IPP, ileal Peyer's patch; JPP, jejunal Peyer's patch; *R*/*S*, replacement over silent mutation ratio

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ileal Peyer's patches (IPP) at days 110–115, about 6 weeks before birth (for a review, see Landsverk et al. (1991)). These precursors have undergone rearrangements of the lambda locus that make use of a relatively small part of the large V lambda (*IGLV*) genomic pool, estimated at 90–100 genes, and of the unique J lambda gene (*IGLJ*) (Reynaud et al., 1991b). Proliferation of these precursors takes place in the IPP follicles during the first 6 months after birth, along with an ongoing hypermutation process that gives rise to a diversified repertoire. In a series of elegant experiments, Reynaud et al. (1995) have shown that this process is largely antigen-independent, and that the hypermutation mechanism by itself appears to be able to introduce replacement mutations preferentially in the complementarity determining regions (CDRs). These conclusions were recently challenged by Jenne et al. (2003), who suggest that the size of the expressed germline pool has been underestimated, and that a significant part of the diversification may be the result of combinatorial mechanisms rather than of hypermutations.

More recently, a study on the kappa locus rearrangements during the fetal stages has shown a slightly different pattern (Hein and Dudler, 1998). Two different J kappa genes (*IGKJ*) rearrange with about 10 V kappa genes (*IGKV*), in a process that involves a rather extensive junctional variability, with very variable CDR3s as a result. This variability is apparent in spleen as early as 60 days of gestation, before the start of JPP and IPP colonization. A decrease in diversity is observed later on, with only two *IGKV* and one *IGKJ* gene used in the sequences expressed in peripheral blood lymphocytes after day 117. Eventually, the adult light chain repertoire is predominantly (over 80%) made of the lambda isotype (Griebel and Ferrari, 1994). This evolution raises the intriguing question of the role of a diversity that is largely restricted to the fetal life of the individual.

While it is generally assumed, by analogy with the chicken (Reynaud et al., 1989, 1994) and the rabbit (Knight and Barrington, 1998; Mage et al., 1999) systems, that very few if any new rearrangement occur in sheep after the start of gut-associated lymphoid tissue colonization by B cell precursors (Reynaud et al., 1997), the limited diversity of the expressed lambda germline pool makes it difficult to test this hypothesis accurately. In order to address this question, we turned therefore to the heavy chain locus, whose D regions may provide an additional discriminating element. We have previously obtained the sequence of six different heavy V genes (*IGHV*) which, in addition to three pseudogenes, probably constitute the majority of the germline pool (Dufour et al., 1996). The heavy chain J locus (*IGHJ*) was also sequenced completely (Dufour and Nau, 1997); it contains two functional *IGHJ* genes only. In the present paper, we analyze and compare the variable region diversity of rearranged genes originating from sheep fetuses, neonatal lambs (4 days old), young (3 months) and adult (over 6 months) animals.

2. Materials and methods

2.1. Preparation of DNA

Samples of IPP tissue from a 144-day-old fetus and from a 4-day-old lamb were a kind gift of Dr. C.A. Reynaud (INSERM U 373, Hôpital Necker, Paris, France). The source of adult DNA was the spleen of two different animals, obtained from two local slaughterhouses. DNA was extracted from these tissues by grinding small fragments to a fine powder in a mortar partially filled with liquid nitrogen, overnight incubation at 37 °C in the presence of 1% SDS and 5 mg/ml Proteinase K, extraction with saturated phenol/chloroform/isoamyl alcohol (10:10:1), and ethanol precipitation. DNA extracted from the leukocytes of two 3 month old lambs was a kind gift of Dr. F. Lantier (INRA, Centre de Tours-Nouzilly, France)

2.2. DNA amplification

PCR amplification of V–D–J genes was performed using the following primers: 5′-CGGGATCCAGGAGTGGTGACT(T/C)TCATCTGC-3′ (LV_H) and 5′-TGAGGAGACGGTGACCAG-3′ (J_H3'). The design of these primers is discussed in Section 3. Amplification was performed in a final volume of 50 μl, with 2.5 U of recombinant polymerase, under the following conditions: 5 min at 94 °C, 35 cycles of 30 s at 60 °C, 30 s at 72 °C, 30 s at 94 °C, 5 min at 60 °C with a final 5 min incubation at 72 °C. PCR products were fractionated on a 1% agarose gel and extracted from the gel using an extraction kit (Qiagen,