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Cloning and characterisation of the primary structure of the sheep lymphocyte function-associated antigen-1 α subunit

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Abstract

The leukocyte integrins play a critical role in a number of cellular adhesive interactions during the immune response. The ovine cDNA encoding CD11a, the predominant α subunit of the β_2-integrin family, was sequenced and compared with the human, bovine and murine sequences. Despite some focal differences, it shares all the main characteristics of its known mammalian homologues. Along with the ovine CD18-encoding cDNA, which is available for a few months, the sequence data provided here will allow the *Ovis aries* β_2-integrin CD11a/CD18 (LFA-1, $\alpha_L\beta 2$) expression in vitro as a tool to examine the specificities of inflammation in the ovine species.

Keywords: Integrin; LFA-1; ICAM; Adhesion; Ovine

1. Introduction

Lymphocyte function-associated antigen-1 (LFA-1, $\alpha_L\beta_2$, CD11a/CD18) is a member of the β_2-integrin subfamily of cell surface receptors. Integrins consist of a 120- to 180-kDa α subunit (CD11a in this case) and a 90- to 110-kDa β subunit that are noncovalently associated single-pass transmembrane proteins (Springer, 1990). The bulk of each integrin subunit is extracellular, where it typically functions as a receptor for extracellular matrix molecules or as a counterreceptor for surface proteins of apposed cells (Hynes, 1992). The heterodimer CD11a/CD18 is expressed on all leukocytes and mediates high affinity adhesion to a variety of cell types that express one or more of the β_2-integrins ligands, intercellular adhesion molecules (ICAM-1 to -5) (Bailly et al., 1995; Gahmberg, 1997; Tian et al., 1997). The adhesion process mediated is a critical step of a wide range of immunological activities, including cytolysis of target cells, cross-interaction and cross-stimulation between lymphocytes, phagocytosis of complement-coated targets, neutrophils clearance from inflamed tissue, and the regulation of leukocyte traffic between the bloodstream and tissues (Dunne et al., 2003; Hogg et al., 2004; Salas et al., 2004; Yan et al., 2004). As the relevance of the ovine model has been well established in such diverse areas as immunology (Mitazaka and Trnka, 1985), haematology (Zanjani et al., 1997), genomic cloning (Fulka et al., 1998) or asthma (Abraham et al., 2000; Bischof et al., 2003; Collie, 2003), increasing our knowledge about ovine β_2 integrins is of great importance to offer new possibilities for research and to provide additional insights into those fields.

The *Ovis aries* CD18 (β_2) subunit has been well characterised (Zecchinon et al., 2004), which is not the case of its partner into the LFA-1 heterodimer, CD11a. The purpose of this paper is to report the cloning and sequencing of a cDNA encoding ovine CD11a, along with a comparative sequence analysis with its human, bovine and murine homologues.

Abbreviations: Bo, bovine; hu, human; ICAM, intercellular adhesion molecule; IDAS, I-domain allosteric site; kan, kanamycin; LFA, lymphocyte function-associated antigen; MIDAS, metal-ion dependent adhesion site; mu, murine; ov, ovine; PBMC, peripheral blood mononuclear cell; PMA, phorbol myristate acetate; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-polymerase chain reaction

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2. Materials and methods

2.1. RNA isolation

Total RNA from phorbol myristate acetate (PMA)-stimulated (25 ng/ml for 15 min) ovine peripheral blood mononuclear cells (PBMC) was extracted with TRIzol (Invitrogen) as described by the manufacturer. The PBMCs were obtained by density gradient centrifugation with Ficoll-Paque Plus (Amersham) and maintained in RPMI 1640 supplemented with 10% foetal bovine serum (Gibco BRL), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37 °C in a 5% CO_2 atmosphere.

2.2. Amplification of cDNA ends

We used SMART rapid amplification of cDNA ends (RACE) technology (Clontech Laboratories Inc.) to obtain ovine CD11a (OvCD11a) 5′- and 3′-ends and reverse transcription-polymerase chain reaction (RT-PCR) to amplify full-length OvCD11a CDS. For first-strand cDNA synthesis, and according to the sequence of BoCD11a available (GenBank no. AY267467), gene-specific primers were designed which were expected to give non overlapping ∼1 kb RACE products: a sense primer for the 3′-RACE PCR: 5′-TGCAATGTRAGCTCTCCCATCTTC-3′ (corresponding to nt 2572–2595) and an antisense primer for the 5′-RACE PCR: 5′-CCGGCCTCCTCTCTGCTCCCCATAG-3′ (nt 1470–1446). Reverse transcription and polymerase chain reactions (PCR) were carried out according to the instruction manual of the SMART RACE cDNA Amplification Kit. The 5′- and 3′-RACE products were gel-purified using the S.N.A.P.™ Gel Purification Kit (Invitrogen), TA-cloned into pCRII-TOPO (Invitrogen) and seeded on kanamycin IPTG plates. Miniprep were obtained from colonies grown in 5 ml LB-Kan broth and the clones were sequenced on the ABI-3730 Genetic Analyzer using the Big Dye terminator chemistry (Applied Biosystems).

2.3. Molecular cloning of full-length cDNA

Total RNA from PMA-stimulated PBMCs was reverse transcribed using Improm II (Promega). The full-length cDNA was then generated by long distance PCR using Advantage 2 polymerase (Clontech Laboratories Inc.) with primers designed from the distal ends of both 5′- and 3′-RACE products: 5′-GTCGCCAGTAAATCCCAAGA-3′ (sense, within the 5′-UTR) and 5′-GCACCTCAATCTCCACCACT-3′ (antisense, 3′UTR). The procedures recommended by the manufacturer were followed, with these cycling parameters: 5 min at 94 °C, then 35 cycles including (i) 30 s at 94 °C, (ii) 30 s at 60.5 °C and (iii) 3 min 30 s at 68 °C, followed by a final extension at 68 °C for 5 min. Resulting PCR products were then processed for sequencing as aforementioned for the RACE products. The CD11a cDNA sequence was deduced from sequences obtained from nine independent clones. Sequence data have been deposited at GenBank under accession no. AY731091 and AY731092.

2.4. Bioinformatics

Primers design was performed with Netprimer (http://www.premierbiosoft.com/netprimer) and Primer 3 (Rozen and Skaletsky, 2000). Nucleotidic sequence and similarity analyses were carried out using, respectively, Chromas v.2.21 (http://www.technelysium.com.au) and BLAST programs (Altschul et al., 1990). Alignment of amino acids sequences were drawn by GeneDoc v.2.6.002 (Nicholas et al., 1997) following the BLOSUM 62 matrix. SignalP v.2.0.b2 (Nielsen et al., 1997) and NetNGlyc v.1.0 (Jensen et al., 2002) provided peptide signal and *N*-glycosylation sites prediction, respectively.

3. Results and discussion

3.1. Characterisation of OvCD11a-encoding cDNA and deduced aa sequence

Two alleles have been identified for the OvCD11a cDNA. The sequence contains ∼4200 bp with an ORF of 3498 (Genbank no. AY731092) or 3495 bp (Genbank no. AY731091) depending on the allele that codes for 1165 or 1164 aa followed by ∼600 bp in the 3′-UTR (Fig. 1). The mature OvCD11a contains a 23-aa putative leader peptide, an extracellular domain of 1061 or 1062 residues (24–1084/1085), a single hydrophobic transmembrane region of 24 residues (1085/1086–1108/1109) and a cytoplasmic tail of 57 residues (Fig. 1). Seven N-linked putative glycosylation sites (Asn-X-Thr/Ser) are present in the extracellular domain (Fig. 2). The mature protein contains 21 cysteine residues, among which one is located into the cytoplasmic tail (Fig. 2). The extracellular domain also contains an inserted (I) domain of 172 amino acids (residues 153–324) quite similar to those found in all the leukocyte integrin α subunits sequenced to date and located between the β sheets 2 and 3 of a seven bladed β-propeller region (Huang et al., 1997). The I domain is homologous with repeated domains found in von Willebrand factor and cartilage matrix protein (Springer, 1990) and can be expressed as an isolated domain. Its three-dimensional structure consists of a five-stranded parallel β-sheet core surrounded on both faces by α-helices, with a short antiparallel strand occurring on one edge of this sheet (Qu and Leahy, 1995). The I domain contains a metal ion dependent adhesion site (MIDAS) (Lee et al., 1995) (residues 159–163, 228, 261) (Fig. 2) and an I-domain allosteric site (IDAS) that plays a functional role in ICAM-1 binding (Huth et al., 2000; Lupher et al., 2001; Lum et al., 2002). Three repeats with a divalent cation binding motif are found at amino acid residues 465–473, 527–535 and 587–595 (Fig. 2). All the conserved cysteines and all but one *N*-glycosylation sites are found out-