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Technical report

Expressed gene sequence of bovine IL23A and IL23R

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ABSTRACT

The cloning and characterization of bovine IL23A and IL23 receptor cDNA from total RNA of PBMC and the genomic organization of the coding sequences are reported. The IL23A partial coding region was found to be 578 nucleotides coded for in 4 exons and shared 84% and 76% identity with human and mouse sequences, respectively. The IL23R complete coding region had 1890 nucleotides coded for in 10 exons and shared 87% and 73% homology with the human and mouse sequences, respectively. Both bovine sequences were more closely related to the human sequences than were mouse sequence. This work was done as part of the U.S. Veterinary Immune Reagent Network whose goal is to develop reagents for investigating diseases in livestock species, poultry and fish.

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IL-23 is a heterodimeric cytokine that is composed of the p19 subunit (its gene referred to as IL23A) and p40 subunit (its gene referred to as IL12B). Another cytokine, IL-12, which is important for the development of IFN γ -producing T_H1 cells, has the same p40 subunit (its gene referred to as IL12B) as IL-23 but paired with a p35 subunit (its gene referred to as IL12A) (Kastelein et al., 2000). Despite these similarities, IL-12 and IL-23 have different functions. IL-12 is essential for antimicrobial responses, particularly for intracellular pathogens (O'Garra and Arai, 2000), and functions as a suppressor of human B cell tumors (Airoidi et al., 2004) whereas IL-23 plays a pivotal role in survival of Th17 cells which produce IL-17, a cytokine involved in the recruitment of neutrophils and inflammatory responses (Veldhoen et al., 2006) and the establishment and maintenance of organ-specific inflammatory autoimmune diseases (Langrish et al., 2005). It has been demonstrated that IL23A-deficient mice are resistant to experimental autoimmune encephalomyelitis (EAE),

collagen-induced arthritis (Cua et al., 2003; Murphy et al., 2003), and inflammatory bowel disease (Langrish et al., 2005), indicating an important role of IL-23 in autoimmune pathogenesis.

IL-12 and IL-23 also differ in that IL-12 acts on naïve T cells while IL-23 acts on memory CD4⁺ T cells (Lankford and Frucht, 2003). This is because the IL-23 receptor-unique subunit (its gene referred to as IL23R), which pairs with IL-12R β 1 (its gene referred to as IL12RB1) to make a functional receptor, is expressed by memory CD4⁺ T cell only (Parham et al., 2002). In contrast, the IL-12R β 2 (its gene referred to as IL12RB2), which also pairs with IL-12R β 1, is expressed by naïve CD4⁺ T cells when activated (Kastelein et al., 2000). IL-23R is also expressed with NK cells (Parham et al., 2002). With regard to veterinary medicine, IL-23 has been associated with inflammatory diseases of importance; transcripts for IL-23 are increased in the lungs of horses exposed to hay dust (Ainsworth et al., 2007). We have cloned the bovine IL-23p19 subunit (IL23A) cDNA and bovine IL-23 receptor (IL23R) cDNA to generate tools to evaluate their roles in inflammatory diseases of cattle and generation of Th17 cells.

To generate the IL23A and IL23R clones, alignments of the published human IL-23 alpha subunit (its gene referred to as human IL23A, GenBank accession no. NM_016584;

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Kastelein et al., 2000) and human IL23R (GenBank accession no. NM_144701; Parham et al., 2002) mRNA sequences with the *Bos taurus* genome build 3.1 were performed using the Nucleotide Basic Local Alignment Search Tool (BLASTN; Altschul et al., 1990). Both sequences aligned with portions of chromosome 3. Specifically, sequence similar to the human IL23A sequence was identified on GenBank contig NW_931537 and described as “similar to putative alpha subunit interleukin 23” while sequence similar to the human IL23R was identified on contig NW_001494804 and described as “similar to putative interleukin 23 receptor.”

Primers flanking the putative coding regions of the predicted bovine IL23A and IL23R sequences were designed based on predicted sequences described above. Primer sequences are as follows: IL23A forward 5'-ATGCTGGGGAACAGAGCTG-3'; IL23A reverse 5'-TAGGGGCTCAGAGTTGCTGC-3'; IL23R forward 5'-ATGAATCAGGT-CACAATTC-3'; IL23R reverse 5'-CTACTTTTCTAAGAGTG-AAATCCG-3. Bovine peripheral blood mononuclear cells (PBMC) were isolated from whole blood as described previously (Rogers et al., 2006) and total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Total RNA was used for cDNA synthesis using the Reverse Transcription System (Promega, Madison, WI) and oligo dT primers. Polymerase chain reaction (PCR) was carried out using PCR Master Mix (Promega) using a final concentration of 2 mM MgCl₂ and final primer concentrations of 200 nM each. Cycling conditions were as follows: 30 s at 95 °C and 1 min at 55 °C for both bovine IL23A and IL23R, and 45 s for bovine IL23A and 2 min for bovine IL23R at 72 °C for the subsequent 35 cycles. PCR products were analyzed via agarose gel electrophoresis, purified using a QIAquick Gel Extraction Kit (Qiagen Sciences, MD) and ligated into the pCR2.1 vector (Invitrogen). Sequencing of cDNA clones was performed commercially (GeneWiz, South Plainfield, NJ) using M13 reverse and T7 forward primers in separate

reactions. All sequence analyses were performed using BioEdit software (Hall, 1999), unless otherwise indicated.

Bovine IL23A and IL23R cDNA sequences were aligned with bovine genomic contigs (Fig. 1) using the program Spidey (Wheelan et al., 2001). The schematic representation of the exon–intron organization shows our results which indicated that bovine IL23A gene included 4 exons while bovine IL23R had 10 exons. This agreed with human IL23A but contrasted with human IL23R which comprise 4 and 11 exons, respectively. The bovine IL23A and IL23R cDNA sequences obtained here were 99.7% and 99.6% homologous to their respective predicted sequences found on bovine genomic contigs NW_931537 and NW_001494804 (GenBank).

Alignment of bovine, human and murine sequences for IL23A coding regions (Fig. 2A) showed that bovine IL23A shares 84% and 76% identity with equivalent human (GenBank accession no. NM_016584, bases 167–736; Kastelein et al., 2000) and mouse (GenBank accession no. NM_031252, bases 113–703; Kastelein et al., 2000) nucleotide sequences, respectively. At the amino acid level, bovine IL23A shared 80% and 64% identity with equivalent human (GenBank accession no. NP_057668) and mouse (GenBank accession no. NP_112542) sequences, respectively. The predicted molecular weight of bovine IL23A gene product (i.e. IL-23p19 subunit), based on the deduced amino acid sequence was found to be 21.3 kDa. Interestingly, the deduced amino acid sequence of bovine IL23A contained conserved residues that comprised helices also found in both equivalent human and mouse sequences (Fig. 2A, shown in gray). In addition, analysis of the bovine IL23A deduced amino acid sequence with Signal-P (Emanuelsson et al., 2007; Bendtsen et al., 2004) predicted cleavage between amino acids G²¹ and R²¹ with 66.1% probability, as has been predicted for the human (Kastelein et al., 2000) but not for mouse (Kastelein et al., 2000) proteins. No other cleavage sites were predicted.

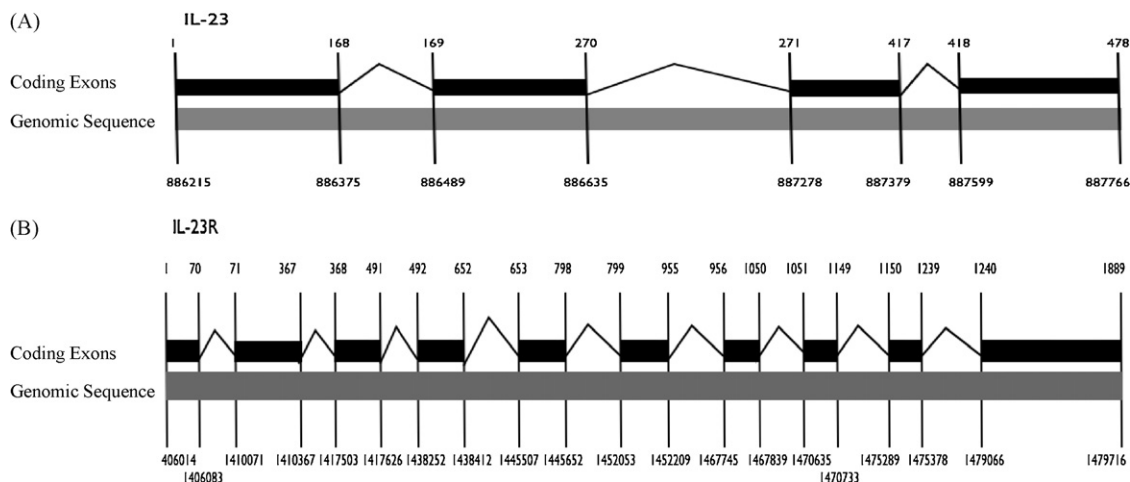


Fig. 1. Schematic representation of the exon–intron organization of (A) bovine IL23A based on the alignment of the IL23A cDNA sequence (indicated in black) reported here (GenBank accession no. EU616677) with bovine genomic sequence (indicated in gray, contig GenBank accession no. NW_931537) and (B) bovine IL23R based on the alignment of the IL23R cDNA sequence (indicated in black) reported here (GenBank accession no. EU616678) with bovine contig GenBank accession no. NW_001494804 (indicated in gray).

A

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bo IL-23 ATGCTGGGAACAGAGCTGTGATGCTGCTGCTGCTACTGCTGCCCTGGACAGCTCAGGGCCGGGCTGTGTGTCAGAGGACAGCAGCCCTGCTTGGACTC 100
hu IL-23 .....G.....A.....T.....-----.....A.A.....C.T.G..G.....C..... 94
mo IL-23 .....ATTG.....A.A.AA...ATG...T-----.....GTCA.....T.....C.TAG.AGT...T...AC...G... 94

bo IL-23 GGGGCCAACAGCTCTCACAGCAACTCTGCATGCTGCGCTGGAGTGCACACC TACCAATGGGACATATGGATCTACCAAGAGAAGAAGGAGGTGATAAGAC 200
hu IL-23 A.T....G....T....A..G.....CA.....T.C..T.G.....C.....-.....G...A..AG... 191
mo IL-23 A.T....G.....T.G.A.T.....A.....AC.....TGC...GC.....A.....T.....AG.A..AG... 194

bo IL-23 TACAGATGATGTCGCCCGTATCCAGTGTGAGGATGGCTGTGATCCACAAGGACTCAGGGACACAGTCACTCTGCTTGCAAAAGAAATTCATCGAGGCCGTC 300
hu IL-23 ...A.....T...A.....GA.....C..C.....T.....G...C..C..AG..T... 291
mo IL-23 ..A.A.A...G.....A.....T.....C.....A.....C...T.....G...C..GC.A..T... 294

bo IL-23 GTTTTTTACGAGAAGCTGCTGGGCTCAGATATTTTCACAGGGGAGCCTTCTCTACTCCCAAATGGCCCTGTGGACCAGCTTCACGCCCTCCATCTGGGCC 400
hu IL-23 A.....T.....A..A.G.....TG.A.....G.....T.....C..... 391
mo IL-23 .C.....TA..C.C...T.A..T..C..C...A.....G.....TG.A...CA...G..A...A...C...A..A. 394

bo IL-23 TCAGGGAAGCTCTTGAGCCCAAGGTCACCACTGGGAACTGAGCAGACTCCAAGCCCTATCCAGCCAGCCATGGCAGCGCCTCTCTCCGCTCTCAA 500
hu IL-23 ...CC...C...TG.....G...C...T.....TC.G.....T.....CT... 491
mo IL-23 ...CC...C.C...AG..A...C.C...G..CC.A...TG..C...TG.G.T.T..T..AG.....C.....TC... 494

bo IL-23 GATCCTTCGAAAGCTCCAGGCCCTTTGTGGCCGTAGTCCCGGGTCTTTGCCACGGAGCAGCAACTCTGA---GCCCTA----- 578
hu IL-23 A.....C.....T...C.....T.....C.....-T...A----- 579
mo IL-23 .....T...A.....CTGA...T..GTGCCAACAGCTTAA 591

bo IL-23 MLGNRAVMLLLLLLPPWTAQGRAVSEDSPPAWTGGQOLSQQLCMLAWSAHLPMGHMDLPREEGGDKTTDDVPRIQCEGDGCPQGLRDNSSCLORIHRLG 100
hu IL-23 ...S.....P.G.....CC...K...T.....PLV.....DEE..N..H..G.....F.....Q... 97
mo IL-23 ...DC...IM.W...VT..L...PRG...D.A...AC...RN...N.A..A...N.L...E.EE.KNN...K...F.....RQ... 98

bo IL-23 VFYEKLLGSDIETGPESSLPNGEVLDOLHASICLRELLQPKGHHWETEOTPSPIPSQEWORLLRLKILRSLOAFVAVARVFAHGAATLSP 192
hu IL-23 F.....DS..G...L...SQ...E...Q..I..LS...F.....* 190
mo IL-23 A..KH..D...K..A..DS.ME...T..L..SQ...ED.FR..Q.M..LSS..Q...P...S.....T..T.....TEPIVPTA* 197
    
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B

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bo IL-23R ATGAATCAGGTCACAATTCATTGGGACGTGGTAATAGCTCTCTACATATTTCTCAGTGGTGTCTATGGAGGATACAAATATAAACTGCTCTGGACACA 100
hu IL-23R .....T.....A.....T.CA.....C..T.....C.....A.....C..... 100
mo IL-23R .....G..CC.....C...GCT.C.T...G.....C..T..TG.GC.....A.....C.....A..C...G.....TG... 100

bo IL-23R TCTGGGTAGAACCTGCCACAATTTTAAAGATGGGTATGAATATCTCTATATATTGCCAAGCAGCAATTAAGAATGCCAACCAAGTAAACTTTATTTTAA 200
hu IL-23R .....A.....G.....G.....C.....G..... 200
mo IL-23R .G.....T..G...GTGA.....C.....C.....G.T.....A...CC...C...G.....G..T...C... 200

bo IL-23R TAAAAATGGCATCAAAGAAGATTTTCATATCACAGAAATCAATAAAACAACAGCTCGCCTTTGGTATAAACTTTGTGGGCCCAAGCCCTTTGTGTG 300
hu IL-23R .....A.....G..T.....G.....A.....T..T.C.A... 300
mo IL-23R .....T.....GA...G.....G..T...G.....GA.....AGG...TC...A..T..T...A.A..C.T 300

bo IL-23R TGTACTGCTGAATGTTCAGATATTTCCAGAGACACTGATATGTGAAAAAGACATTTCTTCTGGATATCCACCAGATGTACCTGACAAAGTAGCCTGTG 400
hu IL-23R ..C.....C..A..C.....A.....G.....C.....G...C...A..T...TG...A... 400
mo IL-23R ..C.....C.T.G.TC...A.....T.....G.....C.....C.....G...CC..CAG...TC.GA.A... 400

bo IL-23R TCATTTATGAATATTTGGCAACATGACTTGTACCTGGAACCTGGGAGGCCACCTACATAGACACAAAGTATGTGGTGTACGTGAAGAGTTAGAGAC 500
hu IL-23R .....A.....C.....TG...A..T.....A..C...AC.T... 500
mo IL-23R .....C.A.....A..C.....A.....A..T.....T..C...A.T...C.T...G... 500

bo IL-23R AGAAGAAGAGCAAGAATACTCACTTCAAGTTACATTAACATCTCCACTGATTCATTGCAAAAGGGCAAGAAGTATTTGGTTGGGTCCAGCTTCAAA 600
hu IL-23R .....C.G.....C..T.....G.....A...GGT...C.....AG...C 600
mo IL-23R .....A..C.....TG.C...C..TG...G.....C..C...GGCA..G.....A.....GTC... 600

bo IL-23R GTTCTGGGCTGGAGAAGTCGAAACACTACAATTCATCTGGAGATATAGTGATACCTTCTGCATCCATTATTTCCAGGGCTGAGGATATAAACTA 700
hu IL-23R .CA..A.....AG..A.....G.....C.....T.....G..G.C...C...A...T..T.C.A...G... 700
mo IL-23R TCC..A.....C..AC.....CG.C...T.....G.....C.....AC..C...CGA... 700

bo IL-23R CAGTGTCCAAGACTGTAATCCACTGGGATAGTCAAAACATCAATGAAAAGTTTCTGTGAAATGAGATACAAAGTACAAACAACTTGGAAACGT 800
hu IL-23R .....C.....CA...TT.T.....A.....G.....G...C...T... 800
mo IL-23R .T..AC.....CA..G.TT...A.A..CA...TATG...G...A.T...G.....ACA...G...GT... 800

bo IL-23R TAAAGAATTTGATACCAATTTTACATACGAGCAACAGTCAGAATCTACTTGCAGCCAAATGCTATGTATTTCAAGTGAATGTCAGAAACAGGT 900
hu IL-23R .....C.....T.T.....CAT..A..C.....C 900
mo IL-23R .....CG.....C...T.TA..G.....C..G...G.CAGC.A...C.....T... 900

bo IL-23R AAAAGTACTGCCAGCCCTGGAGTTCACCCCTTTTTTCATAAACTCCTGAA~----- 951
hu IL-23R .....G.....T.....TG.....A..... 951
mo IL-23R .....GAA.....T.....C.....G.C..CC...T.CC...GAAACTGGTAAAAGAACTGGCAGCCCTGGAGTTCGCCCTTTGTCACC 1000

bo IL-23R ~~~~~~ATAGTTCCTCAGGTCACAATGAAATCATTCCAACATGATACTCAGAAATCTGGACTTCTAATTCCTTCATCTTAAAAAACATCTTAC 1040
    
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Fig. 2. Alignment of bovine IL23A (labeled as IL-23) and IL23R (labeled as IL-23R) expressed sequences with those from human and mouse. Periods indicate identity relative to the bovine sequence and dashes indicate gaps. (A) Comparison of bovine (GenBank accession no. EU616677), human (GenBank accession no. NM_016584; Kastelein et al., 2000) and mouse (GenBank accession no. NM_031252; Kastelein et al., 2000) IL23A nucleotide and deduced amino acid sequences. Helical regions are shaded in gray. (B) Comparison of bovine (GenBank accession no. EU616678), human (GenBank accession no. NM_144701; Moore et al., 2002) and mouse (GenBank accession no. NM_144548; Moore et al., 2002) IL23R nucleotide and deduced amino acid sequences. WSXWS motif residues are shaded in gray.

hu IL-23R	~~~~~.C.....C.....TCA...G.....C..ATG.....G..AAC.G.....C..C.GGG..C.....	1040
mo IL-23R	AAACTTCCCAG.C...T.C.....T..GCA.....~TCC..C..AC.....GATG.AGA.G..C.G...A.A.....C.G.GG...C.G.	1097
bo IL-23R	TTCTGACACACGGAAGACATTGGACTTTTATTGGGAATGGTCTCTTTGCTGTATTGCTGTCTAGTTCATCTTTGATTTGGGATATTTAACAGATCGCCT	1140
hu IL-23RAGG.....A..G.....T.....A...T.....AT.C	1140
mo IL-23R	..A.GT..TCATC.....G.C.....G..CA.C...T..C.GA..T.T..C.....A...	1197
bo IL-23R	CGAACTGGGATTTAAAGAAGAATCTTATGCTAATACCAAAATGGCTCTATGAAGATATTCCTAATATGGAAAACAGTAAAGTTGTGAAAATCCITTCAGG	1240
hu IL-23RG.....T.....G.....T.....A.....C..T.....G..A....	1240
mo IL-23R	...TA..A.....A.G.T...C..A.G.C...G...T.....T..C..T...CA...T..AT..A...	1297
bo IL-23R	AAAGAAATGAGTTTATGAATAACAATTCAGCGAACAGGTCCTATATGTTGATCCGGTGATTACAGAGATA~~GAAATCATCTCCCAGAGAAAAAAC	1337
hu IL-23R	...AT.G...AC.....T.....T..G.....CA.....AAA.....T.CA.....C.C..G...	1340
mo IL-23R	...A.G..TA...GA...G.T...G...T..G...C...G...G...T..CC.....AGT.....TC..C..TG...C.C...	1397
bo IL-23R	CATGGGCTACAGAAGAAAACAATACAGGATGCTGGAGAGAAAAGAGAGCCTGGAGAAATCCTACTACCAGCCTACAGTGTGTTATATTCCTGAT	1437
hu IL-23R	T.CA.A.....G..G.T...~.CC.....C..G...CTA..C.C.A..C..G...T..GA.A.TA.....A.....	1437
mo IL-23R	..CA..AT...AG...GGCTC.....CT...T...C..G...CT.T.CTCTAGG..ATGT..GTCT..AGPT..T.T...G.....C	1497
bo IL-23R	CTCAACTGGGATATAAACCAGATTCAAGTTTCTCCCTGGGGAAACCATCTCAGCAATGATGATGAACAGCTTCCTCAATCTCTGGAACACCAG	1537
hu IL-23RA.....A.....A.....G...A.....G...A.....G...T.TA.....T..CA..TA.....	1537
mo IL-23RA.C.....G.....A.G...CT...A.....T.T...TT..CAGA...G..ACC.TA..TCC..T..GA.CA...	1597
bo IL-23R	CTGATTCCTTAAACTTGGGAACAATGCCAGGTTTAAAAAATATCCCTGATTTTGCTTTTCTGTCTCAAGTCAAAATTCCTAAGCAACACACTATTCT	1637
hu IL-23RG...CA.....T...C.....AC...GC.....CA.....GTG.....T.....GTG.....	1637
mo IL-23R	A..C~.....C..TT.....A..G...C...CA.C..CCAA...CT.....TGGC..T...A...A.....A...	1682
bo IL-23R	TGAAGAAATAAACCCTCAITTTAAATCAAGGAGAATGCAGTCTCCGGACATGCAAAACTCAATAGAGGGGAAACTGCCATGCTCTGGAAGATGCATTA	1737
hu IL-23R	..G...G...A.....T...T...A.....G.....A.....A.....T.....A.....AT.C...	1737
mo IL-23R	..T.....GTG...G.....T..A.T...TT...AA.....G.C...A.....CAG...CG..T..C..AG..AC.C...	1782
bo IL-23R	CTGAATGAAACTATCCAGACAACAACCTGCTGCCTGATGAATTTGCTCCTGTTTGGGGAGCATGAACARAAGAGTTGCCATCTATTAATCTTACTTTC	1837
hu IL-23R	..CC.G.....G..C.....T.....T.G...TG.G.....A...T...	1837
mo IL-23R	..CC.G.....C...CG..G.....T..T.....CA..TTGG...TG...C.....	1882
bo IL-23R	CACAAAATATTTGGAAAGCCACTTCAATCGGATTTCACTCTTAGAAAAGTAG	1890
hu IL-23RA.....G.....	1890
mo IL-23R	...G..CG.....T...G.A.A.....CC.....	1935
bo IL-23R	MNQVTIHWDDVIALYIFPSWCHGGITNINCSGHIWVEPATIFKMGMINSIYQAAIKNCQPSKLYFYKNGIKERPHITRINKTTARLWYNNEVPEQAFVY	100
hu IL-23R	...Q..A.....L.....R..H.....Q.....K..L..H.SM.	100
mo IL-23R	..SHL.LQLH...VL.R...S...DM..GE..Q...V...E..L.H.R.RN.....F..E.D...R...I..KG.S..H.YMH	100
bo IL-23R	CTAECRSYFPETLICGKDISSGYPPDVKVACVIYBYSGNMTCTWNPGRPTIYIDTKYVVVKSLETEEBEQEYLTSYINISTDLQKGGKYLWVQASN	200
hu IL-23R	...PKH.Q.....I..E.T.....A.KL.....H.....Q.....G.....A.200	200
mo IL-23R	...PGH.Q.....H...A.SNLT.....T.K.....I.H.....Q..A...VK.....GSR.....V.200	200
bo IL-23R	VLGMEKSKQLQIHLDDIVIPASIIISRAEDINTIVSKTVIHWDSQTSIEKVSCEMRYKDTTNQTWNVKFEFDNTFYEQQSEFYLPQNPAMYVQVRCQETG	300
hu IL-23R	A...E.....AV...T..A..P..I.Y...T.....A.....V.....E..IK.....	300
mo IL-23R	S...N.Q..HV.....TT.D..P...IVY.K.K.M...F...T...S...A...V...E..DSK.....	300
bo IL-23R	KKYWQPWSSPFFHKTPET~~~~~IVPQVTMKSQPHDTQNSGLLIASIFKKHLSDNKRKIDIGLLGMVFFAVMLSVLSLIGIFNRSI	380
hu IL-23R	..R.....L.....T.....S.A...W...TV...STG.....G.....IV.....I.....F	380
mo IL-23R	..RN.....V.Q.SQETGKRNNQWSSPFFVHQTSQT.S...A...S.EP.KMEM.S.T..RG.PA.G.HQ.....S...L..I..PIF.....	399
bo IL-23R	RTGIKRRIILLIPKWLIEDIPNEMNSKVVKILQERNEFMNNSSEQVLYVDPVITEI-EIILPEEKPMGYKKNNTGCLERKESLEKSLITDQVYIPD	479
hu IL-23RK..N...M...NS.L.....M...K..FI..H..TD.....P..TRDYPQN..FDNT.....	479
mo IL-23R	..I...KV..M.....N.A.L...KSV.E.D.A...A...L...S...SPL.H..TD..E.RL..L..TRDCPLGM.S.SSS.....	499
bo IL-23R	LNTGYPKQISSFLPGGNHLSNDDDETASSILEPPADSLNLGNARFKYPPDFAFVSSTNSLSNTLFLEELNLILNQGECSPDQNSIEGETAMLEEDAL	579
hu IL-23RN...E.S...NN.IT.LT.K..V...DS...P.LQ.H.N...V...I..G..S.....S..I..V.E.T...NDS	579
mo IL-23RV.NVP...LFI.R..RDPTS..TTD.~HF..L.T..N.Q..A..MAL.NK..I.D..C.V...FNSL.IK..RQE..SIV.QSDS	594
bo IL-23R	LNETIPEQTLPLDPEFVSCGLGSMNKELPSINSYFPQNILESHFNRISSLEK* 630	
hu IL-23R	PS.....IV.E.....T.....*	630
mo IL-23R	PS...A...S.....AIG.ED.....V...S...FQ.* 645	

Fig. 2. (Continued).

Alignment of bovine, human and murine sequences for IL23R coding regions (Fig. 2B) showed that bovine IL23R shares 87% and 73% identity with equivalent human (GenBank accession no. NM_144701, bases 86–1975; Parham et al., 2002) and mouse (GenBank accession no. NM_144548, bases 121–2055; Parham et al., 2002) nucleotide sequences, respectively. Based on the deduced amino acid sequence, the predicted molecular weight of bovine IL23R gene product is 150.7 kDa. Similar to bovine IL23A gene product, we found that at the amino acid level bovine IL23R gene product shared 81% and 63% identity with equivalent

human (GenBank accession no. NP_653302) and mouse (GenBank accession no. NP_653131) sequences, respectively. The deduced amino acid sequence of bovine IL23R contained a conserved WSXWS motif also found in equivalent human and mouse sequences (Fig. 2B, shown in gray). Signal-P (Emanuelsson et al., 2007) was used to analyze the deduced amino acid sequence of bovine IL23R and predicted cleavage between amino acids G²³ and G²⁴ with 68.9% probability. This was the only cleavage site predicted and was in agreement with predictions for the human (Kastelein et al., 2000) and mouse (Kastelein et al., 2000) proteins.

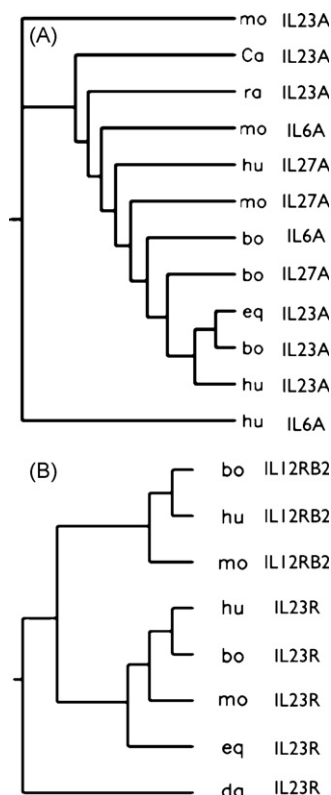


Fig. 3. Cladograms showing relationship of bovine IL23A to those from human, mouse, equine, bat, and rat (A) and bovine IL23R sequences to those from human and mouse (B). Abbreviations are as follows. hu: *Homo sapiens*; mo: *Mus musculus*; bo: *Bos taurus*; eq: *Equus caballus*; ca: *Carollia perspicillata*; ra: *Rattus norvegicus*; da: *Danio rerio*. The cladograms were constructed based on alignments of the coding region sequences. GenBank accession numbers not already referenced in the article are as follows: hu IL6: NM_000600; mo IL6: NM_031168; bo IL6: NM_173923; hu IL27: NM_145659; mo IL27: NM_145636; bo IL27: XM_866578; hu IL23A: NM_016584; mo IL23A: NM_031252; eq IL23A: NM_001082522; ca IL23A: EU223817; ra IL23A: AY05379; hu IL23R: NM_144701; mo IL23R: NM_144548; eq IL23R: XM_001499980; da IL23R: NM_001113506; hu IL12RB2: NM_001559; mo IL12RB2: NM_008354; bo IL12RB2: NM_174645.

Bovine IL23A coding region sequence was compared with sequences encoding other IL-6 helical cytokine family members using the Kitsch program (a component of the PHYLIP package in BioEdit [Felsenstein, 1989; Hall, 1999]). The resulting tree (Fig. 3A), demonstrated that bovine IL23A is more closely related to equine IL23A than to the equivalent human or mouse genes. *Carollia* IL23A sequence was used as an outlier for the generation of the tree (GenBank accession no. EU223817). Bovine IL23R coding region sequence was compared with sequences encoding other WSXWS family members using the Kitsch program. Based on the tree (Fig. 3B), bovine IL23R is more closely related to human IL23R than mouse IL23R is to human. The tree was generated using human and mouse IL12RB2 coding region sequences (GenBank accession no. NM_005535 and GenBank accession no. NM_008354) as outliers. Bovine IL23A and IL23R were also clearly more closely related to human IL23A and IL23R, respectively, than to other members of the helical cytokine and WSXWS

motif families. (Images for Fig. 3 provided through the Bioweb server maintained at the Institut Pasteur, Paris, France.)

The goal of the US-Veterinary Immune Reagent Network (www.vetimm.org) is to address the insufficient availability of reagents required to better understand immunology and infectious disease in livestock, poultry and fish. Bovine IL-23R will be expressed in CHO cells and the protein used for mAb production to evaluate its distribution among cell populations. Bovine IL23A will be expressed with bovine IL12B in yeast and evaluated for bioactivity and used to make mAb. Because of the high level of similarity between bovine and human IL23A, it is predicted that bovine IL23A will function similarly in inflammatory disease processes and generation of Th17 cells in ruminants as has been shown for other species. The availability of the reagents will allow this to be evaluated experimentally.

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