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Cloning and sequencing of the horse and sheep high-affinity IgE receptor α chain cDNA

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Fc ϵ R1, the high-affinity receptor for IgE, is expressed on the surface of mast cells and basophils. It plays an important role in IgE-mediated allergic reactions that are initiated by the binding of the Fc domain of IgE to this receptor and the cross-linking of the receptors in the presence of sensitizing allergens. Fc ϵ R1 is a tetrameric, transmembrane protein with one α , one β , and two γ subunits. The extracellular portion of the α subunit is responsible for binding IgE (for review, see Kinet 1999). This important, high-affinity IgE receptor is also expressed by other cells. For example, the functional expression of Fc ϵ R1 α by dendritic cells enhances IgE-mediated presentation of allergens in humans but not rodents (Shikabi 1999) and platelet-expressed Fc ϵ R1 is associated with allergic release of anaphylactic mediators (Hasegawa et al. 1999). Domesticated animals such as horses are exposed to many of the same environmental allergens encountered by farmers and other individuals suffering from occupational asthma and chronic obstructive pulmonary disease (COPD). Horses exposed to stable dust, for example, suffer from a COPD that involves mast cell activation and IgE responses to fungal allergens (Halliwell et al. 1993; McGorum et al. 1993; Schmalenbach et al. 1998). Similarly, sheep mount potent IgE responses against nematode infections (Kooyman et al. 1997) and are increasingly used in comparative studies of airway allergic responses (Clark et al. 1995).

The aim of the present study was, therefore, to clone and sequence the α subunits of the high-affinity IgE receptors in these two species. This will make possible the expression studies and functional analyses of the role of this high-affinity receptor during naturally induced allergic responses such as horse COPD (Halliwell et al. 1993). The mast cell-derived serine protease, tryptase, in sheep airways (Pemberton et al., 2000) and its functional involvement in airway hyper-responsiveness (Clark et al. 1995) suggest that the role of IgE and its high-affinity receptor will be of considerable comparative interest in this model of allergic airway disease.

Using cells recovered from bronchoalveolar lavage fluid (BALF) for horse, and cultured bone marrow mast cells for sheep, cDNAs encoding the α subunit for the receptors were cloned using RT-PCR. Cell pellets were obtained from BALF taken from horses undergoing treatment for COPD (Halliwell et al. 1993) and were used for the preparation of RNA if the mast cell content was greater than 3%. Sheep bone marrow mast cells were cultured in lymphocyte-conditioned medium with the addition of interleukin-3 and stem cell factor (Macaldowie et al. 1997). Total RNA was extracted from the cells using TRI reagent (Sigma). RT-PCR, cloning, and sequencing were done as described by McAleese and coworkers (1998). cDNA was amplified first using primers H1 and H2 which were based on well-conserved regions in other known high-affinity IgE receptor α chains. Degenerate positions were introduced where there was uncertainty. The 3' ends were amplified by PCR using the primer H3 and an oligo(dT)-anchor primer. For the 5' ends, a 5'3' rapid amplification of cDNA ends kit (Roche Molecular Biochemicals) was used, following the manufacturer's instructions. Total RNA was reverse transcribed using the gene-specific primer H5, and the dA-tailed cDNA was amplified twice by PCR using the 5'dT-anchor primer supplied and the gene-specific primers H6 and H7 for the horse sequence,

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Table 1 Sequences of primers used for amplification of the α chain of the high-affinity IgE receptor for horse and sheep. H1–4 were based on the published sequences for rat and human (Kinet et al. 1987; Kochan et al. 1988). Oligonucleotides were supplied by Cruachem (UK)

H1 Sense	CCTTGRACCCWCCATGGAWTAGAATA
H2 Antisense	TAAYCCTGTGTCCACAGCAAACAGAA
H3 Sense	TGGCTGCTGCTTCAGDCHTCTGCT
H4 Antisense	GATCACCTTGTTRSACATYCCAHTTCYTCC
H5 Antisense	ATCCTTGTAGTAGATCACCTTGAAG
H6 Antisense	TGGCACCTGAGAAGGAGGKWCTTACCCTC
H7 Antisense	AGCAGAGGCCTGAAGGAGCAGCCA
H8 Sense	TRGAGGCCACRGAGRAGATGCCT
H9 Antisense	TGCARAGTTTTCTTGAGSAGTGACA
S7 Antisense	ACCCTCCATCATCACCTCAGCAGA
S8 Sense	CTAAGGCCCTGGATCTCTGCAGCT
S9 Antisense	TTCATGGTTTCATACTTTTATTCAAGTTGT

and H6 and S7 for the sheep sequence. Primer sequences are shown in Table 1.

The full-length cDNA sequences, obtained initially from three overlapping fragments, were confirmed by sequencing complete cDNA clones. These were prepared using primers H8 and H9 for the horse and S8 and S9 for the sheep. A total of 985 bp of horse nucleotide sequence has been obtained, including 768 bp of open reading frame. RNA from two different horses was used and the sequences showed only two base

horse

ctcgtgtggcacctggaggccacagaggagATGCCTGCTCCCATGGGAAGCCCTGCCCTGC
(M) P A P M G S P A L L

TGTGGATAACTTTTCTGCTCTCTCTGATGGCGTGCCAGCAGCCATCCGGAATCTA
W I T F L L F S L D G V P A A I R K S T

CAGTGTCTTGAATCCCCATGGAATAGAATATTTTCGAGGAGAGATGTGACTCTTACAT
V S L N P P W N R I F R G E N V T L T C

GTAATAAGAACAGCCCTTAAAGGCACTCCACTGAGTGGACCTACAACAACACCACTT
N K N K P L K G N S T E W T Y N N T T L

TAGAAGTGACAACCTCAAGTTGAACATCACTAATGCCTCACACCGGAGCAGTGGGAAAT
E V T T S E L A F N I T A S L N S G E Y

ACAGATGTGGAACAATGACTTGAACCTGAGTGAAGCTGTGCACCTAGAAGTTTTCAGTG
R C R N N D L N L S E A V H L E V F S D

ACTGGCTGCTCCTTCAAGCCTCTGCTGAGGAGGTATAGAGGGTAAGGCCCTCGTTCTCA
W L L L Q A S A E E V I E G K A L V L R

GGTCCCGTGGTGAAGGATGGGACGCTCTCAAGGTGATCTACTACAAGGATGGCAAC
C R G W K D W D V F K V I Y Y K D G K P

CCCTCGAGTACTGGTATGAGAACAAAACATCTCCATTGAAAGTGCCACAACAGAAAACA
L E Y W Y E N K N I S I E S A T T E N S

GTGGCACCTATTACTGCGAGGGTGTCTTTAACTTTAAGCGAACAAGTGAACGCTATACCT
G T Y Y C E L A F N F K R T S E R Y T S

CTGATTACCTCAACATTACTGTAAAAAAGCTGAGCAAGCAACGCTACTGGCTACAAT
D Y L N I T V K K A E Q S K R Y W L Q F

TTATTATTCCATTGTTGGTGTGATTCTGTTTGCTGTGGACACAGGATTGTTTGCTCGA
I I P L L V V I L F A V D T G L F V S T

CCCAGCAGCAGTTAACATTTCTCTGAAGATTAAGAGGACCAGGAGAGGCAGAAAACCTTA
Q Q Q L T F L L K I K R T R R G R K L M

TGGACCCCATCTTAAGtgagacccgagaagaactgatgtcactcctcaagaacacctt
D P H P *

tgcaacagcaattttctcctggcagcaattgcttttcagttgtcaaacacagatcat
aatgtacatagaagggtctatgccacggctttgcagaattgcatcattaaactaactag
aactgggttaagtggcatgtaataag

A

sheep

B

ctaaggccctggatctctgcagctcagttggaggccacgagaagATGCCTACTATTCCC
(M) P T I P

ATGGGAGCCCCCTGCCCTGCTGTGGATAGCTCTGCTGCTTTCTCTCCAGATGGCATGTCA
M G A P A L L W I A L L L F S P D G M S

GCAGCCAATGGAAATCTAAGGTGTCTTGAATCCACCATGGAGAGAAATATTTAGAGGA
A A N W K S K V S L N P P W R E I F R G

GAAACTGTGACTCTTACATGTGGTGTGAACAGATCCTCTGAAATGAGTCCCTCTGTGG
E T V T L T C G V N R S S E N E S S V W

ATCCACAATGGAACCACTTTGAAAGAGACAAATCAAGATGGGACATTGTGAAAGCACGC
I H N G T T L K E T N S R W D I V K A R

GTACAGGACAGTGGGAAATACCAATGCCGAATCAAGGATTTGCCATTAGTGAGCCTGTG
V Q D S G K Y Q C R I K G F A I S E P V

TACCTAAACGTATCAGTACTGGTGTGATCCTTCAGGCCTCTGCTGAGGTGATGATGGAG
Y L N V I S D W L I L Q A S A E V M M E

GGTGAGTCCCTCTCTCAGGTGCCAGTGTGAAGAATCTGAATGCCTCAAGGTGATG
G E S L F L R C H S W K N L N A F K V I

TACTACAAAGATAACAGAGCTCTCAAGTACTGGTATGAAACACACAACATCTCCATCACC
Y Y K D N R A L K Y W Y E N H N I S I T

AATGTACAGAAGGGGACAGTGGCATCTATTACTGTGTGGGCAGAATTCAGAGGCTCAAC
N V T E G D S G I Y Y C V G R I Q R L N

TACACCTCTAATAAACTCAAGATTATAGTAAAAAAGCAGTACACTCAAAGCAACTATTTTC
Y T S N K L K I I V K K Q Y T Q S N Y F

TGGCTACAATTTCTTATCCATTGTTGGTGTGATTCTGTTTGTGTGGACACAGGGTTG
W L Q F L I P L L V L I L F V V D T G L

CTTATCTCGACCCAGCAGCAGTTCACGTTACTCTTGAAGATGAAGAGAATCAGAAAACGC
L I S T Q Q Q F T L L L K M K R I R K R

AACAGATTACCGACCCACAGCCTAAACAGATCCCCAGGGGAAGTGAgtcactcctca
N R F T D P Q P K P D P P G N *

agaaacctctgcagtagcagaggttttcggttagtaaacgcttctcagttgtcaaga
cagctcacaaggtacatagaaaaagctcacagctttatagaactgcttcattaaactgat
cgagtggcatgtaataaggaagtgtcaataagcatcattatataagtaagaagaatgagt
agattcatttattagaactctgtaaaagagatatacaacttgtaataaaagtatgaaccat
ga

Fig. 1 Nucleotide and deduced amino acid sequences for horse (A) and sheep (B) Fc ϵ R1 α . Potential N-linked glycosylation sites are underlined, as is the polyadenylation signal for the sheep sequence

changes, one in the coding region and one in the 3' noncoding region. Amino acid 244 in the deduced amino acid sequence was threonine in horse 1 and isoleucine in horse 2. For the sheep α chain, a total of 1082 bp of nucleotide sequence has been obtained which includes 783 bp of open reading frame. These results are shown in Fig. 1.

A comparison of the deduced amino acid sequences for Fc ϵ R1 α for the horse and sheep with those for human, dog, and rodent are shown in Fig. 2. The horse cDNA appears to encode a signal sequence of 24 amino acids and a mature protein of 230 amino acids. For the sheep cDNA, the lengths are 25 and 234 amino acids, respectively. A high degree of identity is seen between the different sequences with many conserved features. The overall identity of the horse α subunit with those from human, sheep, and dog was 65%, 58%, and 57%, respectively. The sheep α subunit showed 60% identity with both the human and dog sequences. Comparison with the rodent sequences showed rather lower identities of between 40 and

	Signal peptide	∇	mature protein	•		•				
horse	MPA-PMGSPA	LLWITFLLFS	LDGVPAAIRK	STVSLNPPWN	RIFRGENVTL	TCNKNKPLKG	NS-TEWTYNN	TTELVTTSSL	NITNASHRSS	GEYRCRNNDL
sheep	M.TI...A...	...AL...	P...MS...NW	...K...R	E...T...	...GV.RSSEN	E...SV.IH.G	...KE.N.RW	D.VK.RVQD	...K.Q...IKGF
human	MAP-A.E..T	...CVAL.F.A	P...L.VPQ	PK...N	...K...	...G.NFFEY	S...K.FH.G	SLS.E.N...	...V...KFED	...K.QHQOV
dog	M...S.G...	...LAL...S	PGVMSSDTL	P...M...	T.LKDD	...TG.NS.EV	D...AV.LH...	...WQE...R	D.NK.QIQD	...E.RS
rat	MDT.GS.	R.CLALV.I	GVMLT.TQ	V...D...I	...LT.DK...	I...G.NSSQM	...K.IH.D	SISN.KS.HW	V.VS.TIQD	...K.I.QKQGF
mouse	MVT.RS.	Q.CLALLFM	...VILT.TE	...VLT.D...I	...T...K...	S.YG.NH.QM	...T.K.IH.G	VS...NS.H	V.VS.TVQD	...K.I.QKQ
horse	NLSEAVHLEV	FSDWLLQAS	AEVIEGKAL	VLCRCGWKDW	DVFKVIYYKD	GKPLEYWYEN	KNISIESATT	ENSGTYCYEG	AFNFKRTSER	YTSYLNITV
sheep	AI...P.Y.N	I...I...	...VMM...ES	F...HS...NL	NA...	NRA.K...	H...TNVTE	GD...I...V	----RIQRLN	...NK.K.I
human	E...P.Y...	...I...	...V.M...QP	F...H...RN	...Y...	EA.K...	H...TN.TV	D...T...	----VQQLD	E.EP...
dog	I...DP.Y.T	TE...I...	...NV.M...ESF	LI...HS...NL	RLT...T...	I...IR...	F...SNVT	K...N.S.S	----QIQQKG	...KV...I
rat	YK.KP.Y.N	MQE...S	...DV.LDNGSF	DI...S...K	K.H...	DIAPK.S.DS	N...RK.TF	ND...S.H.T	YL----KVE	CK...KFS.A
mouse	FK.KP.Y.N	TQ...T	DMILVHGSF	DI...H...N	N.R...N	DHAFN.S.S	-PV...RE.TL	ND...T.H.K	YLR----QVE	E...KFR.A
horse	KKA-EQSKRY	-WLQFIPL	VVILFAVDTG	LFVSTQQQLT	FLLKIKRTRR	GRKLMDPHP*				
sheep	...QYT...NYF	...L...	...L...V...	...L...F...	...L...M...IRK	RNRFT...Q.K	PDPPGN*			
human	I...PRE...	...F...	...I...	...V...	...K...	FR.LN...K	PNPKNN*			
dog	...EPTKQNK	SG...L...	...I...	...K...	...V...Q...	...K	NK...E.G	KN*		
rat	V.DYTIIE--	R...L.F.S	A...	...WF...HK.FE	SI...QK.GK	...K.KG*				
mouse	V...YKCK--	Y...L.F...	A...	...LL...EE.FK	SV...E.QK.GK	YK.VETELT				

Fig. 2 Comparison of the deduced amino acid sequences of horse and sheep FcεR1α with those of other species. The horse sequence is aligned with that obtained for sheep, human (X06948; Kochan et al. 1988), dog (D16413; Goitsuka et al. 1999), mouse (J05018; Ra et al. 1989), and rat (J03606; Kinet et al. 1987). Sequence gaps resulting from the optimization of alignments are indicated by *hyphens*. Residues identical with those of the horse sequence are indicated by *dots* (∇ N-terminus of the mature protein, **** transmembrane region, • conserved cysteine residue)

50%. The horse and sheep sequences both have the four conserved cysteine residues thought to be involved in disulfide bridges, and the transmembrane regions are virtually identical with those of the other species. Human FcεR1α has seven N-linked glycosylation sites and is known to be highly glycosylated (Garman et al. 1998). The horse and sheep α chains have eight and six potential sites, respectively (Fig. 1), so may be glycosylated to a similar degree.

These studies confirm that BALF cells/mast cells from horse and sheep express message encoding genes for FcεR1α that have a closer identity with the equivalent human subunit than with rodent FcεR1α. It will be interesting therefore to determine whether, in these domesticated species, the α subunit, like its human homologue, serves the dual function of activating mast cells, basophils, and platelets, and of enhancing the presentation of allergens by dendritic cells.

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