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Molecular cloning of cat interleukin-12

Received: 9 September 1996 / Revised: 2 October

The recently discovered interleukin-12 (IL-12) is a heterodimeric 70 000 M_r cytokine composed of a disulphide-bonded 35 000 M_r (p35) subunit and a 40 000 M_r (p40) subunit. Produced mainly by activated monocytes/macrophages and B cells, it enhances NK/LAK cell cytolytic activity and stimulates cytotoxic T-cell responses and IFN- γ production (for review see Trinchieri 1995). It is therefore a key regulatory molecule in T-helper-cell type-1 (Th-1)-driven immune responses that are considered necessary for the elimination of intracellular pathogens (Scott and Kaufman 1991). In vivo administered IL-12 has powerful antitumor and antimetastatic activity (Brunda et al. 1993; Zou et al. 1995) and is a promising vaccine adjuvant (Afonso et al. 1994; Schijns et al. 1995; Wynn et al. 1995). Due to its inhibitory effects on IL-4 and IgE synthesis, IL-12 may be considered in the prevention of IgE-mediated allergic conditions (Kiniwa et al. 1992).

We are particularly interested in the immune pathogenesis and prevention of a coronaviral disease in cats, feline infectious peritonitis (FIP). Since clinical observations suggest that humoral responses are associated with its fatal course, while cell-mediated immunity is assumed to be protective, and likely afforded by a type-1 cytokine response, we focused on feline IL-12. However, nothing is known about the physiological role of IL-12 in cats. Since it is likely species-specific we decided to clone cat IL-12.

We have cloned a cDNA containing the coding sequence of cat IL-12 by the rapid amplification of cDNA ends (RACE) protocol (Frohman et al. 1988). The mRNA of cat peripheral blood-derived mononuclear lymphocytes, stimulated for 4–24 h with either 0.0075% (wt/vol) of *Staphylococcus aureus* (Pansorbin Calbiochem-Behring Co., La Jolla, CA), or 1–10 μ g lipopolysaccharide (LPS; Difco Laboratories, Detroit, MI) was reverse transcribed into cDNA, using a 35 base oligonucleotide primer containing 17 dT residues and an adaptor sequence. The cat IL-12 p35 and p40 cDNA were amplified by a first polymerase chain reaction (PCR) using a sense primer based on regions of the dog, cattle, and human IL-12 p35 cDNA (5'-CAG-TGCCGGCTCAGCATGTG-3'), and p40 cDNA (5'-ATGCATCCT-CAGCAGTTGG-3') and the antisense adaptor primer (5'-GACTC-

GAGTCGACATCG-3'). Conditions for PCR have been described (Frohman et al. 1988). In a subsequent semi-nested PCR the cat p35 and p40 cDNAs were amplified using the same sense primers and antisense primers that were predominantly based on sequences of dog IL-12 p35 or p40 cDNA (Belke and Buettner; GenBank accession numbers U49085 and U49100, respectively); for p35 (5'-CTAG-GAAGCATTTCAGATAGCTC-3') and for p40 (5'-ATCCTGGGGGTG-GAACCTAAC-3'). The PCR products were cloned into plasmid pGEM-T (Promega, Madison, WI) or pNoTa/T7 (5 prime \rightarrow 3 prime, Inc. Boulder, CO) and sequenced. The 685 base pair (bp) cat IL-12 p35 cDNA sequence that was identical in at least two independently isolated clones contains an open reading frame encoding a 222 amino acid protein (Fig. 1A). The 1006 bp cat IL-12 p40 cDNA sequence that was identical in three independently isolated clones contains an open reading frame encoding a 329 amino acid protein (Fig. 1B). Comparison of the predicted amino acid sequence revealed that the cat IL-12 p35 protein shares 90.5%, 81.5%, 85.1% and 55.4% identity with the dog, cattle, human, and mouse IL-12 p35 chain, respectively (Fig. 2A), while the predicted cat IL-12 p40 protein shares 92.1%, 84.8%, 84.2%, and 68.1% identity with the dog, cattle, human, and mouse IL-12 p40 chain, respectively (Fig. 2B). The availability of large amounts of recombinant cat IL-12, and specific antibodies raised against it, will facilitates studies on the (patho)physiological role of this cytokine in the cat. It would be of interest to determine IL-12's immunomodulatory role in tumor-bearing and allergic cats as well as in cats infected with the feline infectious peritonitis virus (FIPV), the feline immunodeficiency virus (FIV), and the feline leukaemia virus (FeLV), and to study its adjuvant activity in vaccines against these viruses.

Acknowledgments This work was supported by a grant from Virbac (Carros, France).

The nucleotide sequence data presented in this paper have been submitted to the EMBL, GenBank, and DDBJ nucleotide sequence databases and have been assigned the accession numbers Y07761 and Y07762

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A

[illegible]

B

1	A	T	G	C	A	T	C	C	T	C	A	G	C	T	T	G	V	T	A	C	G	T	L	V	T	L	T	G	C	T			
17	A	C	A	P	C	T	C	C	C	L	T	M	A	A	G	C	T	T	G	A	A	T	G	G	A	A	C	K	A	A	T		
33	G	T	A	G	C	A	T	C	C	C	T	C	A	G	C	T	C	C	C	C	T	C	C	C	G	A	A	E	M	A	T		
49	V	E	G	L	C	D	T	C	T	G	H	W	H	P	A	T	D	A	C	P	C	G	G	A	E	M	A	A	T	G	T		
65	T	C	N	T	A	T	C	T	P	E	E	E	G	D	A	T	D	I	T	W	T	T	S	D	S	D	T	S	D	C	A		
81	A	C	C	T	G	C	A	A	T	C	G	A	A	A	G	A	C	A	T	C	A	C	C	T	A	C	C	T	C	T	C	A	
97	S	S	S	E	E	V	L	C	T	A	G	C	T	C	G	K	A	T	L	T	I	Q	W	A	C	C	A	C	C	A	A		
113	A	G	C	A	G	A	A	T	C	T	A	G	C	T	C	T	G	C	T	C	A	C	C	A	C	C	A	C	C	A	A	A	
129	E	F	A	A	G	A	C	A	G	C	T	A	G	C	T	C	H	K	A	A	C	A	A	G	G	A	G	G	C	G	T	T	
145	L	S	H	A	G	C	A	G	C	T	C	L	T	C	T	A	H	K	A	A	A	A	A	G	A	A	T	G	A	A	T	T	
161	G	G	C	S	T	C	T	C	C	C	C	C	A	A	G	C	T	F	G	G	A	C	A	A	T	L	S	T	C	A	A	A	
177	A	A	E	G	K	V	R	V	D	A	C	N	A	R	G	G	A	G	A	A	E	K	A	A	A	A	A	A	A	A	A	A	
193	E	C	Q	T	C	A	G	G	S	A	C	P	A	A	A	G	E	K	A	E	K	A	A	A	A	A	A	A	A	A	A	A	
209	I	Z	V	T	C	G	T	V	D	A	I	H	K	L	K	A	L	K	A	E	Y	E	N	A	A	A	A	A	A	A	A	A	
225	A	T	T	A	A	C	T	G	T	G	T	G	A	C	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
241	A	C	C	A	G	T	T	T	T	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
257	N	L	L	Q	L	K	P	L	K	N	S	R	S	R	H	V	E	V	E	C	C	A	A	A	A	A	A	A	A	A	A	A	
273	A	A	C	C	T	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
289	W	E	V	P	C	T	T	W	S	T	W	S	T	H	S	Y	F	S	Y	F	S	Y	F	S	Y	F	S	Y	F	S	Y	F	
305	T	G	G	A	T	A	C	C	T	A	C	C	A	C	C	A	C	C	A	C	C	A	C	C	A	C	C	A	C	C	A	C	C
321	A	G	C	A	T	C	C	T	C	A	G	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T	C	C	A
337	A	C	A	T	C	C	T	C	A	G	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T
353	A	C	A	T	C	C	T	C	A	G	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T
369	A	C	A	T	C	C	T	C	A	G	C	A	T	C	C	A	T	C	C	A	T	C	C	A	T	C	C						

A

cat	MCPPFSGLLIV	TLIVLHMLHD	HLWLSANLP	FTPSPFQFQC	LHSGTLLIRA
cat	-----	-----S-----	-----S-----	AS-----I-----	-----N-----
cat	-----L-S-----	I-----H-P-----	-----G-S-----	T-A-----R-S-----	-D-Y-----N-----
human	-----A-S-----	A-T-----D**-----	-----V-A-----D-----P-----	P-R-----N-----	-----
mouse	-QS-Y-FL-A-T-A-***	-----	-V-I-VP	SGPAR***	-SQ-RN-KT
cat	ISNTLQKARQ	LEFYESTCTG	EIDHEDIKTD	KTSTVEACL	LELWMSNCEL
cat	V-----	-DYIP-----	-----	-----	-----AT-----
human	W-M-----	-P-----	-----	-----	-----K-----
mouse	TDDMKYK-E	K-KH-A-KD	-----R-----	Q-LKT-----	-----HK-----
cat	ASREISLITN	CGSLASKRDS	PMTTLCLSGI	YEDLKWYQV	FMANAKLIM
cat	-----	-----G-A-----	-----V-----	-----M-----	-----
cattle	-----T-P-----	H-----G-----	-----R-----	-----H-----	Q-----
human	N-----T-P-----	-----	-MA-----	-----	Q-T-----A-QN
mouse	-T-T-ST-R	-PQ-----	L-M-----G-----	-----T-----	-----
cat	DPKRIIFLQD	NMLTAIDML	QALNVNSFV	PONSLEPPED	FYKTKIKICI
cat	-----	A-A-----	-----F-K-----	-----	-----
cattle	-----	-----	-----P-D-----	-----P-K-L-----	-----
human	-----	-AV-----M-----	-----F-E-----K-----	-----	-----
mouse	HNHQ-I-T-K	G-G-----	M-S-H-GE-L-R	KPPVGA-A	P-R-VN-----
cat	LLHAFIRIRV	TINRMSLYN	AS-----	-----	-----
cat	-----	-D-----S-----	-----	-----	-----
cattle	-----	-D-----S-----	-----	-----	-----
human	-----	-D-----V-----	-----	-----	-----
mouse	-----ST-V-----	-V-G-S	SA-----	-----	-----

B

cat	MHPQQLVIAW	LSVLVLAPPL	MAIWELEKNV	YVVELDWHPD	AGEMVVLTC
dog	F--S--S--S	F--S--S--S	I--I--D--	--S--S--S	--S--S--S
cattle	--VS-F--S	--SI-V-M--	--S--S--S	--Y--Y--	--T--T--
human	-CH--S--F--P--S--	--V--K-D--	--X--X--	--Y--Y--	--T--T--
mouse	-C-K-E-K-T-S	FAT--VS--	-M--D--	-V-T--	-T-L--
cat	NPEEDDITW	TSPQSEVLG	SGKLTIQVR	EPADAGQVTC	HKGGEVLNS
dog	H--S--S--S	-A-S--S--	--G--G--	--K--K--	--R--R--
cattle	D--D--G--L	--L--L--	--G--G--	--A--A--	--R--R--
human	D--D--G--L	--L--L--	--G--G--	--A--A--	--R--R--
mouse	--S--S--S	--RG-I-E	--T--L--	--L--L--	--T--T--
cat	FLLIHKIKEDG	IWSTDILREK	KESKNNIPLR	CEAKNYSGRF	TGWLMTAIST
dog	--S--S--S	--K--K--	--S--S--	--S--S--	--S--S--
cattle	L--L--L--	--P--T--R--	--S--S--	--K--K--	--S--S--
human	L--L--L--	--P--T--R--	--S--S--	--K--K--	--S--S--
mouse	H--L--N--	--E--KNF	--P--T--	--P--P--	--S--VORM
cat	DLKFTVSSSR	GSBDQEVTC	GAATLSAEKV	RVDNRQYKKY	TVECCQBSGS
dog	--S--S--S	--R--R--	--L--L--	--SLEH-E-N--	--S--S--
cattle	-T-S--S--	-S-P-S-R-G	--M--S--	-G-K-E-B--	-S-S--D--
human	--NI--S--	-S-P-S-R-A	-M-S--	-TL-Q--E-P--	-S-S--DVT--
mouse	--S--S--S	--R--R--	--L--L--	--SLEH-E-N--	--S--S--
cat	PAABESLIEP	VVUDAHLKRL	YENYTSFPI	RDIIKPEEPK	NLIQLKPLKNS
dog	--S--S--S	--R--R--	--L--L--	--SLEH-E-N--	--S--S--
cattle	L--L--L--	--P--T--R--	--S--S--	--K--K--	--S--S--
human	L--L--L--	--P--T--R--	--S--S--	--K--K--	--S--S--
mouse	-T-T--T--	-LALR-QQN-	--ST--	--M--M--	--M--M--
cat	RHVEVSWEYFP	DTWSTPHSYF	SLTFGQVOVQ	KNNRKK****	****KDRLSV
dog	--S--S--S	--R--R--	--L--L--	--SLEH-E-N--	--S--S--
cattle	-Q--Q--Q--	--K--K--	--S--S--	--K--K--	--S--S--
human	-Q--Q--Q--	--K--K--	--S--S--	--K--K--	--S--S--
mouse	*Q--Q--S	--K-F-R-I-R	--KEKM-ETGC	--CBNG--GAPL-	--CBNG--GAPL-
cat	DKTSKQVUCH	KDAKIRVQAR	DRYYSSSSDN	WASVCS****	****
dog	--S--S--S	--R--R--	--L--L--	--SLEH-E-N--	--S--S--
cattle	L--L--L--	--P--T--R--	--S--S--	--K--K--	--S--S--
human	L--L--L--	--P--T--R--	--S--S--	--K--K--	--S--S--
mouse	E--TE--Q--	-GNGVC--Q	--N--C--C--	-C-C-P-KVR-	-C-C-P-KVR-

Fig. 2A, B Alignment of amino acid sequences of cat, dog, cattle, human, and mouse IL-12 **A** p35 and **B** p40 proteins as deduced from their cDNA sequences. **A** *dash* (–) indicates similarity to the cat sequence. An *asterisk* (*) indicates absence of a corresponding amino acid

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