Molecular Cloning of Feline Interleukin-6 cDNA

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ABSTRACT. We cloned and sequenced feline interleukin-6 (IL-6) cDNA using the polymerase chain reaction (PCR) technique to obtain information on regulation of the IL-6 gene. The feline IL-6 cDNA cloned in this study was 752 bp long and included all the amino acid coding region. At the amino acid level, the overall identities of the feline IL-6 to the human and murine counterparts were 58% and 39%, respectively. After transfection with the expression vector containing the cDNA, CRFK cells produced biologically active proteins that showed hybridoma growth promoting activity.—key words: cDNA interleukin-6, feline, polymerase chain reaction.


IL-6 has been identified as an important cytokine involved in regulation of inflammatory and immune responses to infection or injury [16]. It has many biological functions such as stimulation of immunoglobulin (Ig) synthesis [9,21], regulation of acute phase protein synthesis by hepatocytes [5], stimulation of T lymphocyte growth and differentiation [19,26] and stimulation of hemopoietic stem cells [13]. It is produced by various types of lymphoid and non-lymphoid cells on various types of stimulation, such as by T cells stimulated with mitogens [11], B cells stimulated with IL-4 or PMA [12], monocytes stimulated with LPS [11, 20], and fibroblasts stimulated with poly(rI),poly(rC), IL-1, platelet-derived growth factor, TNF, FCS, or LPS [17, 27, 29]. IL-6 has also been shown to be involved in the immunopathology of various diseases in humans. It is an autocrine growth factor for multiple myeloma [15]. The unregulated, constitutive production of IL-6 may induce polyclonal B cell activation leading to hyper-gammaglobulinemia and autoantibody production in certain tumor-bearing patients [8]. The unregulated expression of IL-6 mRNA may also be involved in the pathogenesis of rheumatoid arthritis [7] and Castleman’s disease [30].

Moreover, there are reports that human immunodeficiency virus (HIV) induces IL-6 production by monocytes [22] and B cells [3] in vitro and that HIV-infected individuals show elevated levels of plasma IL-6 and IL-6 production by monocytes [2, 4, 10]. We recently found that overproduction of IL-6 was associated with the pathogenesis of feline infectious peritonitis, which is associated with remarkable hyper-gammaglobulinemia[6], and that the overproduction of IL-6 is also seen in feline immunodeficiency virus (FIV)-infected cats [24].

Because of the potential pathologic role of IL-6 in feline viral diseases, it is important to investigate the mechanism regulating IL-6 expression. In this paper, as a first step in understanding the regulation of the IL-6 expression in a feline immune system, we describe the cloning and sequencing of feline IL-6 cDNA.

MATERIALS AND METHODS

Preparation of cDNA: A normal Japanese domestic cat kept for experimental purposes was anesthetized and injected intravenously with 1 mg/kg of LPS (Serotype 055:B5; Sigma Chemical Co., St. Louis, MO). Three hours later, the mandibular lymph nodes were removed and immediately frozen in liquid nitrogen and mRNA was extracted from 1 g of frozen tissue with a Fast Track mRNA Isolation kit (Invitrogen Co., San Diego, CA). Reverse transcription of 1 μg of mRNA was performed with a cDNA Synthesis kit (Pharmacia, Uppsala, Sweden) and the cDNA was dissolved in 150 μl of TE buffer.

Polymerase chain reaction (PCR) amplification: PCR primers were based on the sequences conserved between human and murine IL-6 [9, 28]. The primer sequences were 5’-AAGCTCTATCTCCCTCCCTAG-3’ (primer AS) and 5’-AAAAATATTAATTAGTGTTC-3’ (primer FR), complementary to the sequences upstream and downstream of the coding sequence of IL-6, respectively. By using these primers, an 810-base pair (bp) fragment including the whole amino acid coding region is expected to be amplified. The cDNA was amplified by PCR in a volume of 100 μl, using primers AS and FR (0.4 μM each), Taq polymerase (1.5 units), and the reagents and protocol recommended by the manufacturer (Perkin-Elmer/Cetus, Norwalk, CT). Amplification was achieved by 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min) and polymerization (72°C, 1–5 min, gradually extended). Then 10 μl of this PCR product was again amplified by the same procedure. The resulting product was electrophoresed in 2% low melting point agarose gel and the amplified DNA band of approximately 800 bp was extracted from the gel. The gel-purified 800-bp DNA treated with the DNA polymerase I (Klenow fragment) (Toyobo, Osaka, Japan) was cloned into the dephosphorylated Sma I site of Bluescript SK (+) (Stratagene, La Jolla, CA). Escherichia coli, DH5α cells (BRL, Gaithersburg, MD) were transformed with the ligation
mixture and plated onto 2X TY agar plates containing 50 
μg/ml of ampicillin, 36 μg/ml of 5-bromo-4-chloro-3-
indolyl β-D-galactoside and 40 μg/ml of isopropyl β-D-
thiogalactoside. Plasmid DNA was prepared from 7 
independent white colonies by either the boiling method 
or with a QIAGEN plasmid kit (QIAGEN, Studio City, 
CA). The DNA inserts of these 7 clones were further 
screened by PCR using another primer pair. This primer 
pair (sense primer, 1S, 5'-GCTTCGATCCTAGTTGCCT-
TC-3'; antisense primer 8R, 5’-CTGACGCCAATGCTT
CTGTG-3') has been confirmed to amplify a 480-bp DNA 
fragment inside the S10-bp AS-FR fragment.

Nucleotide sequence analysis: Two independent clones, 
AF-11 and AF-13 containing feline IL-6 cDNA were 
sequenced. Both strands of the inserts in the plasmids 
were sequenced by the dyeodeoxy chain termination 
method using Sequenase Ver. 2.0 (United States Biochemicals, 
Cleveland, OH).

Expression of IL-6 cDNA: A PstI-KpnI fragment of 
clone AF-13 was inserted into a PstI-KpnI site of the 
expression-cloning vector, pcDL-SRa296[25]. Samples of 15 
μg of either pcDL-SRa296 containing feline IL-6 
cDNA (pcDL-SRa296-IL-6) or the original pcDL-
SRa296 were transfected into CRFK cells by use of 
Lipofectin Reagent (BRL, Gaithersburg, MD). These 
transfectants were cultured in RPMI 1640 containing 10% 
FCS and 50 μg of gentamycin, and the culture supernatants 
were harvested for assay of IL-6 activity.

IL-6 assay: IL-6 concentrations in the culture supernatants 
were determined using the IL-6-responsive rat 
mouse hybridoma cell clone, B3B1, as described previously 
[23]. The proliferation of B3B1 cells was not stimulated by 
other cytokines such as IL-1, IL-2, IL-3, or G-CSF. 
Human rIL-6 was used to construct a standard curve. 
The activity of human rIL-6 was estimated to be 5 × 10^6 
units/mg[1]. The number of growing cells was determined 
by hexosaminidase assay as described by Landegren[18].

RESULTS AND DISCUSSION

Isolation of a cDNA clone of feline IL-6: Using 
LPS-stimulated feline lymph node cDNA as a template, 
we performed PCR amplification with AS and FR primers 
for isolation of a cDNA clone of feline IL-6. 
Electrophoresis of the PCR products of agarose gel gave 3 
distinct bands of about 800 bp. These 3 bands were 
extracted from the gel and cloned into Bluescript SK(+) 
plasmid vector. Seven independent clones derived from 
the bands of about 800 bp were screened for the presence 
of IL-6 cDNA by PCR amplification with an inner primer 
pair, 1S and 8R, which could amplify a band of 
approximately 500 bp. The result showed that the expected 
band was amplified in 2 clones, AF-11 and AF-13.

Nucleotide sequence of feline IL-6 cDNA: The nucleotide 
sequences of the AF-11 and AF-13 inserts were determined 
on both DNA strands. The nucleotide sequences of 
these two clones were identical. So, we used the 
AF-13 clone for further experiments. The cDNA insert of 
the AF-13 clone contains a single large open reading 
frame. In this frame, the initiator ATG is followed by 207 
codons before the termination triplet TAA. The nucleotide 
and deduced amino acid sequences are shown in 
Fig. 1. The sequenced region of the cDNA corresponds to 
nucleotides 24-787 from the 5'-end of the mature form 
human IL-6 mRNA [9]. The deduced amino acid 
sequence of the cDNA indicated that feline IL-6 would consist 
of 208 amino acid residues before removal of a signal 
peptide and the calculated molecular weight of this 
encoded protein is 20, 515.2. The NH2-terminal region of 
the mature feline IL-6 protein is not known, so we can not 
determine the exact boundary of the signal peptide of feline 
IL-6.

Figure 2 shows the alignment of the deduced amino acid 
sequence of feline IL-6 compared with those of the human 
and murine IL-6 sequences. At the amino acid level, the 
overall identities of the feline IL-6 with the human and 
murine counterparts are 58% and 39%, respectively. In 
regions that are highly conserved between human and 
murine IL-6 [28], the sequence of feline IL-6 is also well 
conserved. In particular, 4 cysteine residues at positions 
68, 74, 97, and 107 of feline IL-6 are found at the same 
positions in human and murine IL-6 (Fig.2). This result 
suggests that the cysteine-rich middle region of the mature 
protein may be important for IL-6 activity, although

![Image of a cDNA clone of feline IL-6](image_url)

**Fig. 1.** Nucleotide sequence and deduced amino acid 
sequence of the feline IL-6 cDNA. Numbers above and below 
the sequence show positions of nucleotides and amino acids, 
respectively. These numbers refer to the S' end of the coding 
region. Underlined sequences indicate the regions of oligo

nucleotide primers (AS and FR) used in the PCR. The 
nucleotide sequence data reported in this paper will appear 
in the DDBJ, EMBL and GenBank Nucleotide Sequence 
Databases with the following accession number D13227.
Fig. 2. Comparison of amino acid sequences of feline IL-6 with those of human and murine IL-6. Numbers above sequence are amino acid positions in feline IL-6, starting at the putative N-terminal methionine residue. Dots indicate identities with the feline IL-6 sequence and dashes indicate gaps induced for alignment. Asterisks indicate cysteine residues conserved in the sequences. The overall identities of the feline IL-6 amino acid sequence with those of the human and murine counterparts are 58% and 39%, respectively.

Jambou et al. [14] reported that a cysteine-free recombinant IL-6 that was chemically synthesized on the basis of the human IL-6 cDNA sequence was also biologically active.

Expression of feline IL-6 in CRFK cells: To determine whether molecularly cloned feline IL-6 cDNA encodes biologically active protein, we cloned the feline IL-6 cDNA into a transient mammalian expression vector pCDL-SRa296 to yield pCDLSRa296-IL-6. CRFK cells were transfected with this pCDL-SRa296-IL-6 by the Lipofectin method, and the culture supernatants were tested for activity to induce proliferation of IL-6 responsive hybridoma cells. As shown in Fig. 3, after transfection with pCDL-SRa296-IL-6, the culture supernatants showed significant IL-6 activity and the maximal production of IL-6 was observed at 24 hours after transfection. On the other hand, the supernatants of CRFK cells transfected with control vectors showed no IL-6 activity. This result indicates that AF-13 clone encodes biologically active feline IL-6.

The cDNA and the expression vector of feline IL-6 obtained in this study will be very useful for better understanding of the involvement of IL-6 in feline immune-mediated diseases.

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