Cloning of cDNAs Encoding Multiple Subtypes of Feline Interferon-α from the Feline Epithelial Cell Line

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NOTE Immunology

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Interferon (IFN) has been found to be a substance with antiviral activity [7, 12]. IFN has multiple functions, including anti-microbial, anti-cell proliferational, and immuno-modulating activity [1]. Two types of IFNs have been identified, type I and type II. Type I IFN includes IFN-α, IFN-β, IFN-ω, IFN-δ, and IFN-τ. Novel type IFN-κ has also recently been reported [9]. Type II IFN includes only IFN-γ. Multiple subtypes of IFN-α have been detected in many mammalian species, and individual genes have been cloned [14]. In human, all of the IFN-α genes make up a cluster [6].

cDNA encoding feline interferon (FeIFN) (GeneBank accession number E02521) was cloned by Nakamura et al. for the first time from a feline T-cell line [13]. Its mature protein had 171 amino acids, and they called it FeIFN-α because it had five more amino acids than ordinary IFN-α [11, 16]. cDNAs encoding FeIFN-α of ordinary size as well as longer size were recently cloned from feline lymph node cells by Wanderling et al. [17], and it was found that the longer one had five continuous amino acid insertion sequences unique to only FeIFN-α. INF-α are primarily produced by lymphoid cells [8]. Mouse fibroblast cells and peritoneal macrophages produced mainly IFN-β with a small amount of IFN-α [10]. The characteristics of IFN-α with respect to species-specificity and acid stability have been found to be produced by a feline epithelial cell line named CrFK (data not shown). The epithelial cells are not thought to be the source of IFN-α, so we attempted to clone FeIFN-α cDNA from CrFK cells.

CrFK cells were stimulated with the B1 strain of Newcastle disease virus at MOI 30 for 6 hr. Total RNA s were extracted from stimulated cells using ISOGEN (Nippon Gene, Tokyo, Japan). cDNA was synthesized from mRNA in the presence of oligo dT and AMV Reverse Transcriptase XL (TaKaRa Bio. Inc., Shiga, Japan) and used as a template to clone feline IFN-α cDNA. The primers used for amplification were derived from GeneBank (accession Nos.: J00210, 146204, M10952).

The forward primer 5-GCGGATCCATCTTCCAGGATCCCCGATG-3 and the reverse primer 5-CGAAGCTTTGAGAGAATCATTTCC-3, were used; the BamHI and HindIII restriction sites are indicated as bold letters. PCR was performed with 100 µl in the presence of 1 µl of cDNA, 0.5 µM quantities of each primer, and 2.5 U of Taq DNA polymerase (TaKaRa Taq™) for 30 cycles as follows: 30 sec at 94°C, 30 sec at 60°C, 90 sec at 72°C. The final product was cloned into the Bam HI-HindIII site of pUC18 using the DNA ligation kit ver. 2 (TaKaRa Bio. Inc., Shiga, Japan). The recombinant plasmids were transformed into E. coli strain XL1-blue. Plasmid DNA isolation was performed according to the manufacturer’s protocols using a GFX Micro Plasmid Prep Kit (Amersham Biosciences). The nucleotide sequencing was carried out with a DNA Sequencing Kit [Big Dye Terminator Cycle Sequencing Reaction, Applied Biosystems INC. (ABI); Foster City, Ca, U.S.A.] and an ABI PRISM 310 Genetic Analyzer (ABI) according to the manufacturer’s instructions. Computer-assisted sequence analysis was carried out with a Genetyx-Mac Ver. 10, software package (Genetyx Inc., Tokyo, Japan).

Eight different subtypes of FeIFN-α cDNA were cloned. The sequences available as Gene Bank entries were designated FeIFN-α7: AB094996, FeIFN-α 8: AB094997, FeIFN-α9: AB094998, FeIFN-α10: AB094999, FeIFN-α11: AB095000, FeIFN-α12: AB095001, FeIFN-α13: AB095002, and FeIFN-α14: AB095003. Open reading frames of the above were 567 bps (FeIFN-α13 and 14), 570 bps (FeIFN-α8, 9, 10, 11, 12 and 14), and 585 bps (FeIFN-
α, β, γ). The predicted amino acid sequences of the N-terminal signal were from residues 1–23. The predicted lengths of the mature peptides were 165 (FeIFN-α13, FeIFN-α14), 166 (FeIFN-ω1 to FeIFN-ω8) amino acids. The full length of almost all mammalian IFN-αs was 166 amino acids. The homology of nucleotides and amino acids among all 15 FeIFN-α subtypes, including one previously reported subtype (Gene Bank acc. Nos. FeIFN-ω1: AY117395, FeIFN-ω2: AY117394, FeIFN-ω3: AY117393, FeIFN-ω5: AY117392, FeIFN-ω6: AY117391, FeIFN-ω7: E02521), are shown in Table 1. The homology between FeIFN-α subtypes ranged from 94.8 to 100%. The similarity of amino acids existed at 101 different positions, and 86 of them were conserved in all or either of the subtypes with five amino acid insertions. IFN-α and IFN-ω were found to have a 6-amino acid carboxyl tail. It is presumed that six extra codons were created by the duplication from a IFN-α gene [14]. It is possible that five inserted codons were created by duplication of the FeIFN-α gene because a consensus sequence of amino acids and nucleotides of FeIFN-α was conserved in all or either of the subtypes with five inserted codons, and there were no differences in the magnitude of variation between FeIFN-α subtypes of ordinary size and those that are longer. Evolutionists think that multigene families do not persist for long by chance, and that superfluous members would either be rapidly eliminated or converted to pseudogenes unless they conferred some selective advantages [3, 5]. There are multiple IFN-α gene loci in humans, but only one is functional and others are pseudogenes [2]. Since only one cDNA of long-chain FeIFN-α has been cloned, it is presumed that six amino acid insertions. IFN-ω and IFN-τ are thought to benefit the species [14]. Different subtypes of HuIFN-α reveal differential antiviral activities [15].

ACKNOWLEDGMENT. The authors would like to thank Dr. Teiiti Arao for his kind help with checking the language in this paper.

REFERENCES

Table 1. Homology (%) of amino acids (upper line) and nucleotides (lower line) among each FeIFN-α subtypes

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REFERENCES
CLONING OF FELINE IFN-α SUBTYPE GENES

Fig. 1. Amino acids sequence homology among the FeIFN-α subtypes. Consensus sequence are shown as FeIFN-α. FeIFN-α1, 2, 3, 5, 6, and E02521 are the subtypes already reported. Residue 1-23 are signal sequence.

FeIFN-α MALKSSFLVALVALGNCNSVCSLGCFLPQTHGLLLRALTLLGQMRRLPDASSCQKDRNDFPA 60
FeIFN-α1 ..........................................................S ........................................ 60
FeIFN-α2 .......................................................... ........................................ 60
FeIFN-α3 .......................................................... ........................................ 60
FeIFN-α5 .......................................................... ........................................ 60
FeIFN-α6 .......................................................... ........................................ 60
FeIFN-α7 .......................................................... ........................................ 60
FeIFN-α8 .......................................................... ........................................ 60
FeIFN-α9 .......................................................... ........................................ 60
FeIFN-α10 .......................................................... ........................................ 60
FeIFN-α11 .......................................................... ........................................ 60
FeIFN-α12 .......................................................... ........................................ 60
FeIFN-α13 .......................................................... ........................................ 60
FeIFN-α14 .......................................................... ........................................ 60
FeIFN-ω .......................................................... ........................................ 60

FeIFN-α FPQDVFGGDSHKAKQLSVMVHTNQKIPHEFCTEASSSAWNTLLEEPFCGDLRQLRKL 120
FeIFN-α1 .......................................................... ........................................ 120
FeIFN-α2 .......................................................... ........................................ 120
FeIFN-α3 .......................................................... ........................................ 120
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FeIFN-α11 .......................................................... ........................................ 120
FeIFN-α12 .......................................................... ........................................ 120
FeIFN-α13 .......................................................... ........................................ 120
FeIFN-α14 .......................................................... ........................................ 120
FeIFN-ω .......................................................... ........................................ 120

FeIFN-α EACVQEOVGEAEPLTNED-----SIRNYFQRLSLLLQERKKYSPCAWEIVRAEMRSLY 175
FeIFN-α1 .......................................................... ........................................ 175
FeIFN-α2 .......................................................... ........................................ 175
FeIFN-α3 .......................................................... ........................................ 175
FeIFN-α5 .......................................................... ........................................ 175
FeIFN-α6 .......................................................... ........................................ 175
FeIFN-α7 .......................................................... ........................................ 175
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FeIFN-α11 .......................................................... ........................................ 175
FeIFN-α12 .......................................................... ........................................ 175
FeIFN-α13 .......................................................... ........................................ 175
FeIFN-α14 .......................................................... ........................................ 175
FeIFN-ω .......................................................... ........................................ 175

FeIFN-α YSSTALQKRLRSEK 189
FeIFN-α1 .......................................................... ........................................ 189
FeIFN-α2 .......................................................... ........................................ 189
FeIFN-α3 .......................................................... ........................................ 189
FeIFN-α5 .......................................................... ........................................ 189
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FeIFN-α12 .......................................................... ........................................ 189
FeIFN-α13 .......................................................... ........................................ 188
FeIFN-α14 .......................................................... ........................................ 188
FeIFN-ω .......................................................... ........................................ 194

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