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Molecular cloning and expression of cat interferon-γ

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Interferon-γ (IFN-γ) has been extensively studied in the mouse system. Produced by activated T cells and natural killer (NK) cells, this lymphokine possesses antiviral activity and exerts pleiotropic immunomodulatory activities, including activation of macrophages and NK cells and enhancement of major histocompatibility complex class I and II expression (for a review see Trinchieri and Perussia 1985). It is a key regulatory molecule in the T-helper cell type-1 (Th-1)-driven immune responses that are necessary for the elimination of intracellular pathogens (Scott and Kaufman 1991; Wang et al. 1994). IFN-γ inhibits the production of IgG1 and IgE but stimulates IgG2a production in vitro (Snapper and Paul 1987) and in vivo (Schijns et al. 1994a). In mouse models recombinant IFN-γ has adjuvant activity in malaria, *Leishmania*, and viral vaccines (Playfair and De Souza 1987; Scott 1991; Schijns et al. 1994b).

Nothing is known about the physiological role of IFN-γ from cats. Recombinant dog IFN-γ (Devos et al. 1992)

Fig. 1 Nucleotide sequence of cat *IFNG* cDNA and deduced amino acid sequence

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1                   M   N   Y   T   S   F   I   F   A   F   Q   L   C   I
1   TCT GAA ACG ATG AAT TAC ACA AGT TTT ATT TTC GCT TTC CAG CTT TGC ATA

15  I   L   C   S   S   G   Y   Y   C   Q   A   M   F   F   K   E   I
52  ATT TTG TGT TCT TCT GGT TAT TAC TGT CAG GCC ATG TTT TTT AAA GAA ATA

32  E   E   L   K   G   Y   F   N   A   S   N   P   D   V   A   D   G
103 GAA GAG CTA AAG GGA TAT TTT AAT GCA AGT AAT CCA GAT GTA GCA GAT GGT

49  G   S   L   F   V   D   I   L   K   N   W   K   E   E   S   D   K
154 GGG TCG CTT TTC GTA GAC ATT TTG AAG AAC TGG AAA GAG GAG AGT GAT AAA

66  T   I   I   Q   S   Q   I   V   S   F   Y   L   K   M   F   E   N
205 ACA ATA ATT CAA AGC CAA ATT GTC TCC TTC TAC CTG AAA ATG TTT GAA AAC

83  L   K   D   D   D   Q   R   I   Q   R   S   M   D   T   I   K   E
256 CTG AAA GAT GAT GAC CAG CGC ATT CAA AGG AGC ATG GAC ACC ATC AAG GAA

100 D   M   L   D   K   L   L   N   T   S   S   S   K   R   D   D   F
307 GAC ATG CTT GAT AAG TTG TTA AAT ACC AGC TCC AGT AAA CGG GAT GAC TTC

117 L   K   L   I   Q   I   P   V   N   D   L   Q   V   Q   R   K   A
358 CTC AAG CTG ATT CAA ATC CCT GTG AAT GAT CTG CAG GTC CAG CGC AAA GCA

134 I   N   E   L   F   K   V   M   N   D   L   S   P   R   S   N   L
409 ATA AAT GAA CTC TTC AAA GTG ATG AAT GAT CTC TCA CCA AGA TCT AAC CTG

151 R   K   R   K   R   S   Q   N   L   F   R   G   R   R   A   S   K
460 AGG AAG CGG AAA AGG AGC CAG AAT CTG TTT CGA GGC CGT AGA GCA TCG AAA

168 *
511 TAA TGG TCA TCC TGC CTG CAA TAT TTG AAT TTA
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The nucleotide sequence data presented in this paper have been submitted to the EMBL nucleotide database and have been assigned the accession number X86972

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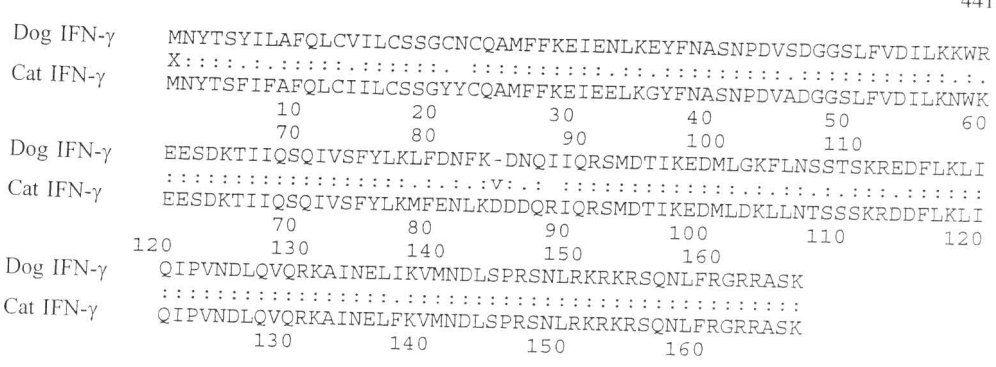

Fig. 2 Alignment of cat and dog amino acid sequences of IFN-γ as deduced from their cDNA sequences. At position 85 cat IFN-γ has an additional asparagine (D). Symbols: *dots* = conservative amino acid substitution, *colon* = conserved residue, *v* = additional residue

exerts no antiviral activity in cat cells (Schijns and coworkers, unpublished observation), despite the phylogenetic relationship between these species. Because of its species specificity we decided to clone cat IFN-γ; we are particularly interested in determining the immunomodulatory role of endogenous and recombinant cat IFN-γ in cats infected with the feline infectious peritonitis virus (FIPV), the feline immunodeficiency virus (FIV), and the feline leukaemia virus (FeLV), and in studying the adjuvant activity of IFN-γ in vaccines against these viruses.

We have cloned a cDNA containing the coding sequence of cat IFN-γ (cat *IFNG*) by reverse transcriptase-polymerase chain reaction on mRNA of concanavalin-A stimulated cat mononuclear lymphocytes, using primers based on conserved regions of the dog *IFNG* gene (Devos et al. 1992). The 543 base pair cat *IFNG* cDNA, which was identical in two independently isolated clones, contains an open reading frame encoding for a 167 amino acid protein with a predicted M_r 17000 (Fig. 1). Hydrophobicity analysis suggests that the first 23 amino acids form the signal peptide that is cleaved from the precursor polypeptide behind the cysteine at position 23. The remaining 144 amino acids would constitute the mature protein. Alignment of the nucleotide sequence of cat and dog *IFNG* revealed a similarity between the coding regions of 89.1%. At the amino acid level there is similarity between 145/167 amino acids (86.8% identity, Fig. 2). Transient eukaryotic expression of the cloned cDNA using the vaccinia virus/phage T7 RNA polymerase system (Fuerst et al. 1986) yielded antiviral activity in a vesicular stomatitis virus inhibition assay on Crandell feline kidney (Crfk) cells and feline catus whole fetus (fcwf) cells. This activity was not observed using control plasmids or dog (MDCK) cells. The availability of large amounts of recombinant cat IFN-γ and specific antibodies raised against it will enable studies to be made of the (patho)physiological role of this lymphokine in the cat.

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