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Molecular cloning of cat interleukin-4

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Interleukin-4 (IL-4), a lymphokine produced mainly by activated T cells and mast cells, is a key regulatory molecule in T-helper cell type-2 (Th-2)-driven immune responses that are considered necessary for the elimination of extracellular pathogens (Urban et al. 1991; Scott and Kaufman 1991). Mice treated with IL-4 neutralizing antibodies (Finkelman et al. 1990), as well as mice bearing a defective *IL-4* gene (Kühn et al. 1991), fail to produce IgE and show reduced serum levels of IgG1. This indicates a pathophysiological role for IL-4 in the generation of certain disease states, like hyper-IgE syndrome or IgE-mediated allergic conditions (Finkelman et al. 1990; Tepper et al. 1990). IL-4-deficient mice have strongly reduced Th2 cytokine responses (Kopf et al. 1993). Due to its inhibitory effects on IFN-γ, IL-4 is considered to down-regulate Th1-associated immune responses necessary for the control of many intracellular pathogens. Recently, it has been shown that neutralization of IL-4 at the time of immunization with inactivated respiratory syncytial virus improves Th1 immune responses and reduces illness against challenge infection (Tang and Graham 1994). Single amino acid substitutions in the human IL-4 molecule result in powerful antagonists with high receptor binding affinity but markedly decreased signal generation (Kruse et al. 1992).

Nothing is known about the physiological role of IL-4 in cats. Since it is likely species-specific we decided to clone cat IL-4; we are particularly interested in determining its immunomodulatory role in allergic cats and in cats infected with feline infectious peritonitis virus (FIPV), feline immunodeficiency virus (FIV), and feline leukaemia virus (FeLV), and to study the adjuvant activity of IL-4 antagonists in vaccines against these viruses.

The nucleotide sequence data reported in this paper have been submitted to the EMBL nucleotide sequence database and have been assigned the accession number X87408

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  1       M   G   L   T   Y   Q   L   I   P   A   L   V   C   L   L
  1  TTA ATG GGT CTC ACC TAC CAA CTG ATT CCA GCT CTG GTC TGC TTA CTA

 16   A   F   T   S   T   F   V   H   G   Q   N   F   N   N   T   L
 49  GCA TTT ACC AGC ACC TTC GTC CAC GGC CAG AAC TTC AAT AAT ACG TTG

 32   K   E   I   I   K   T   L   N   I   L   T   A   R   N   D   S
 97  AAA GAG ATC ATC AAA ACG TTG AAC ATC CTC ACA GCG AGA AAC GAC TCG

 48   C   M   E   L   T   V   M   D   V   L   A   A   P   K   N   T
145  TGC ATG GAG CTG ACC GTC ATG GAC GTC TTG GCA GCC CCT AAG AAC ACA

 64   S   D   K   E   I   F   C   R   A   T   T   V   L   R   Q   I
193  AGT GAC AAG GAA ATC TTC TGC AGA GCC ACA ACC GTG CTC CGG CAG ATC

 80   Y   T   H   H   N   C   S   T   K   F   L   K   G   L   D   R
241  TAT ACA CAT CAC AAC TGC TCC ACC AAA TTC CTC AAA GGA CTC GAC AGG

 96   N   L   S   S   M   A   N   R   T   C   S   V   N   E   V   K
289  AAC CTC AGC AGC ATG GCA AAC AGG ACC TGT TCT GTG AAT GAA GTC AAG

112   K   C   T   L   K   D   F   L   E   R   L   K   A   I   M   Q
337  AAG TGT ACA CTG AAA GAC TTC TTG GAA AGG CTA AAA GCG ATC ATG CAA

128   K   K   Y   S   K   H   *
385  AAG AAA TAC TCA AAG CAC TGA AGC TGA ATA TCT TAA TTT ATG AGT TTT

433  TTA ATT ACT TTA TTT TAA AAA TAT TTA TAT ATT TAT AAT TCA TTA TAA

481  AAT AAA GTA TAT GTA GAA CCC GAA AAA AAA AAA AAA AAA
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Fig. 1 Nucleotide sequence of cat IL-4 cDNA and deduced amino acid sequence

We have cloned a cDNA containing the coding sequence of cat IL-4 by the "rapid amplification of cDNA ends" (RACE) protocol (Frohman et al. 1988). The mRNA of concanavalin-A-stimulated cat mononuclear lymphocytes was reverse transcribed into cDNA, using a 35-base oligonucleotide primer containing 17 dT residues and an adaptor sequence. The cat IL-4 cDNA was amplified by polymerase chain reaction (PCR) using a sense primer based on conserved regions of the human, pig, and cattle *IL-4* gene (5′-TATTAATGGGTCTCACCTACCA-3′) and the antisense adaptor primer (5′-GACTCGAGTCGACATCG-3′). Conditions for PCR have been described (Frohman et al. 1988). The PCR products were cloned into plasmid pGEM-T and sequenced. The 522 base pair feline IL-4 cDNA sequence, which was identical in three independently isolated clones, contains an open reading frame encoding a 133 amino acid protein (Fig. 1). By analogy with human IL-4, cleavage of the precursor peptide probably occurs after Gly24. Comparison of the predicted amino acid sequence revealed that cat IL-4 shares 72%, 67%, 66%, and 50% identity with pig, cattle, deer, and human IL-4, respectively (Fig. 2). The

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Cat     MGLTYQLIPA LVCLLAFTST FVHGQNFNNT LKEIIKTLNI LTARNDSCME
Pig     ----S----T ------C--N ----HKCDI- -Q-------- ----KN----
Cattle  ---------V -----VC--H ----HKCDI- -A-------- --T-KN----
Deer    ----S----V ------C--H ----HKCDI- -E-------- ----KN----
Human   ----S--L-P -FF---CAGN ----HKCDI- -Q-------S --EQKTL-T-

Cat     LTVMDVLAAP KNTSDKEIFC RATTVLRQIY THHN..... .........
Pig     -PGD--F--- E--RE--T-- --S----H-- R--T..... .........
Cattle  -P-A--F--- ---TE--T-- -VGIE--R-- RS-T..... .........
Deer    -P-A--F--- ---TE--TL- --GIE--R-- RS-T..... .........
Human   ---T-IF--S ---TE--T-- --A-----F- SH-EKDTRCL GATAQQFHRH

Cat     .CSTKFLKGL DRNLSSMAN. RTCSVNEVK. .KCTLKDFLE RLKAIMQKKY
Pig     .-MKSL-S-- ---------. M----H-A-. .-S------- ---T--KE--
Cattle  .-LN---G-- ----N-L-S. K------A-T STS----L-- ---T--KE--
Deer    .-LNR--SR- -----GL-S. K------A-T STS---NL-- ---T--KE--
Human   KQLIR---R- ----WGL-GL NS-P-K-AN. .QS--EN--- ---T--RE--

Cat     SKH*.
Pig     --C*.
Cattle  --C*.
Deer    --C*.
Human   --CSS*
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Fig. 2 Alignment of amino acid sequences of cat, pig, cattle, deer, and human IL-4 as deduced from their cDNA sequences. A *dash* indicates similarity to the cat sequence. A *point* indicates absence of a corresponding amino acid

availability of large amounts of recombinant cat IL-4 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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