Indian Journal of Biotechnology
Vol 7, April 2008, pp 195-199

Comparison of nucleotide and amino acids sequence of *Nilgai* (*Boselaphus tragocamelus*) interleukin-18 (IL-18) with other ruminants

Dhanjit Kumar Das[1], Mohini Saini[1*], Devendra Swarup[1] and Praveen Kumar Gupta[2]
[1]Centre for Wildlife, [2]Division of Veterinary Biotechnology, Indian Veterinary Research Institute, Izatnagar 243 122, India

Received 18 April; revised 26 July 2007; accepted 10 October 2007

Interleukin-18, a component of innate immunity with potent interferon-γ-inducing activity, is not well characterized in wild ruminants. In this study, IL-18 cDNA of *Nilgai* (*Boselaphus tragocamelus*) was cloned and characterized. The nucleotide sequence was 582 bp long and contained its entire open reading frame encoding 193 amino acid residues. The sequence analysis indicated seven nucleotide substitutions with reported buffalo sequence showing 98.5% similarity, 97.8% with cattle and 97.6% with sheep. The *Nilgai* IL-18 cDNA included a putative cleavage site of IL-1β-converting enzyme (ICE) and IL-1 signature-like sequence identified in human and cat IL-18 cDNA. Both nucleotide as well as amino acid sequence similarity shows that the cloned sequence was closer to buffalo IL-18 sequence.

Keywords: inerleukin-18, *Boselaphus tragocamelus*, cloning, cDNA sequence, nilgai

Introduction

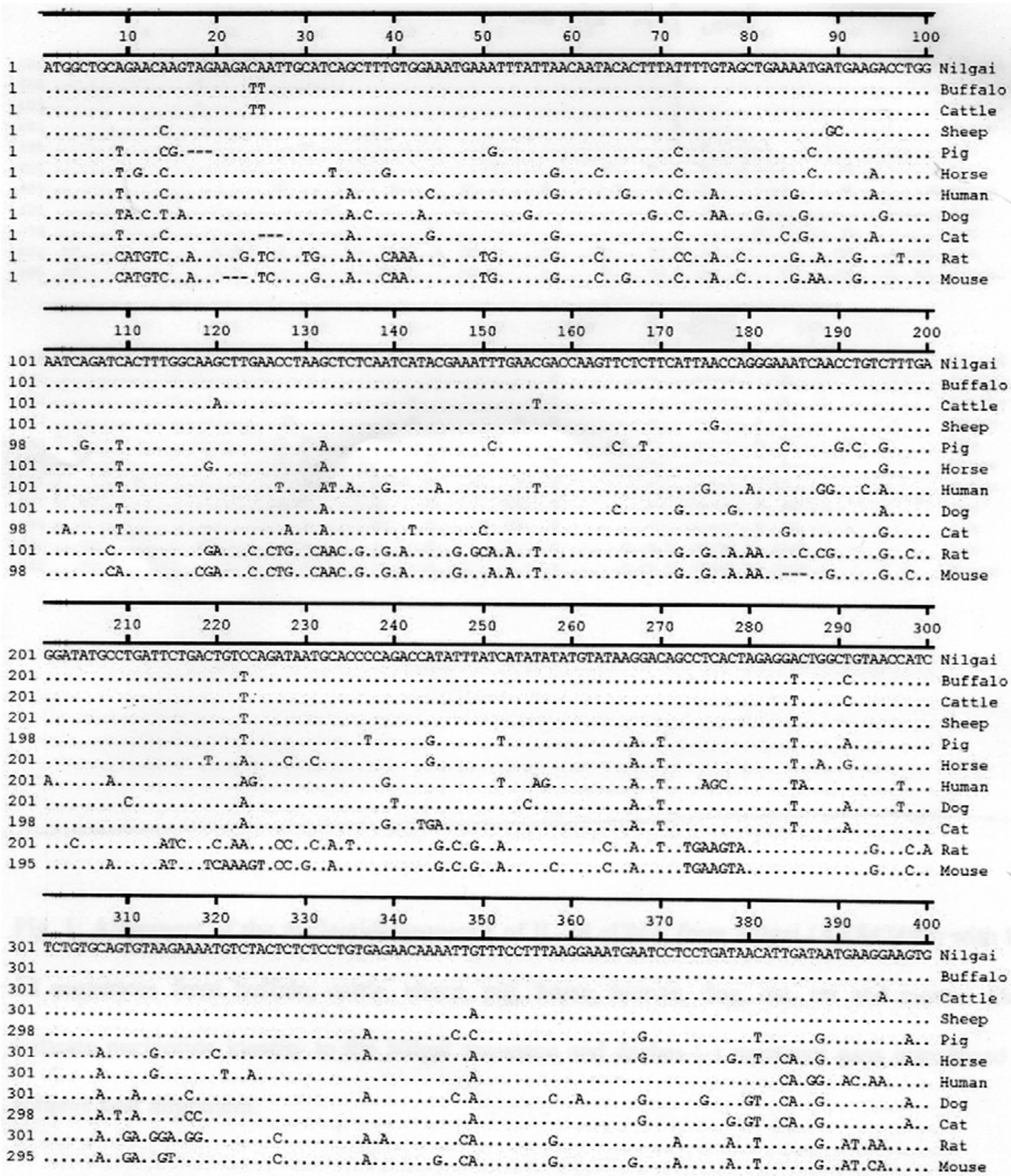
Interleukin-18 (IL-18), a proinflammatory cytokine was originally identified in mouse liver as an interferon gamma (IFN-γ) inducing factor (IGIF)[1]. This cytokine has structural and functional similarities to IL-1 and IL-12, respectively[2-3]. IL-18 produced in a variety of cells, including tissue macrophages such as Kuffer cells, epithelial cells of the intestine and airways, articular chondrocytes and keratinocytes[4-8]. It is also expressed in Payer's patches and in mesenteric lymph nodes[9]. The primary functions of IL-18 are attributed to its induction of IFN-γ in the presence of IL-12, IL-2 and granulocyte monocyte-colony stimulating factor (GM-CSF) and Th1 cell proliferation[10-11]. IL-18 acts synergistically with IL-12 to induce IFN- γ secretion, in part by the upregulation of IL-18 receptors by IL-12[12]. The range of IL-18 activities reported to date suggests that this cytokine plays an important regulatory role in cell-mediated immunity against foreign pathogens and perhaps against tumour cells. IL-18 has been cloned and characterized in mouse[13], human[14], dog[15-16], horse[18] and chicken[19]. However, this interleukin is not yet characterized in wild ruminants. In this study, we report the IL-18 sequence of *Nilgai* (*Boselaphus tragocamelus*), a wild ruminant of Bovidae family and its comparison with that of humans and domestic laboratory animals.

*Author for correspondence:
Tel: 91-581-2301638
E-mail: mohini@ivri.up.nic.in or praveenmohini@rediffmail.com

Materials and Methods

To amplify cDNA, peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque 1077 (Sigma, MO) and stimulated with concanavalin A (Con A) at a concentration of 10 μg mL^{-1} for 20 h at 37°C in a humidified incubator with 5% CO_2. Total cellular RNAs were isolated using Trizol LS (Life Technologies, NY) following the manufacturer instructions. The first strand cDNA was synthesized using oligo (dT) primer and the gene for IL-18 was amplified from cDNA using specific oligonucleotide primers (Forward 5´-ATGGTGCAGAACAAGTAGA-3´, Reverse 5´-TAGTGCTGGTTTTGAACAGT-3´), designed based on the consensus sequence of the other ruminant species viz. cattle (GenBank Accession No. NM_174091) and buffalo (AY394479). A polymerase chain reaction (PCR) was performed using cDNA along with forward and reverse primers (50 pmol each), 200 pmol DNTPs, 1.5 m*M* $MgCl_2$ and 3U of *Taq* DNA polymerase. The amplification cycle consisted of 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and amplification at 72°C for 1 min. The amplified product of 582 bp was cloned into pTZ57R vector (MBI Fermentas, Maryland) and sequenced with 17 universal primers using the ABI PRISM 377 Version 3.0 DNA sequencer (Applied Biosystems, CA).

The 582 bp amplified product contained the whole open reading frame (ORF) for IL-18 and its amino acids sequence was deduced using DNA Star software (Lasergene, Madison, WI) and this sequence of IL-18 was submitted to GenBank (Accession No AY842499). The sequence was analysed with available nucleotide and amino acid sequences of other species viz. cattle (*Bos Taurus*). (GenBank Accession No. NM_174091), buffalo (*Bubalus bubalis*) (AY394479), sheep (AJ401033), pig (U68701), horse (Y11131), human (NM_001562), dog (NM_001003169), cat (Y13923), rat (AY258448) and mouse (NM_008360) using DNA Star software (Fig. 1). Phylogeny tree, based on evolutionary distances, was constructed from nucleotide and amino acid sequences using Treecon software (http://iubio.bio.Indiana.edu/soft/molbio/evolve/draw/treecon).



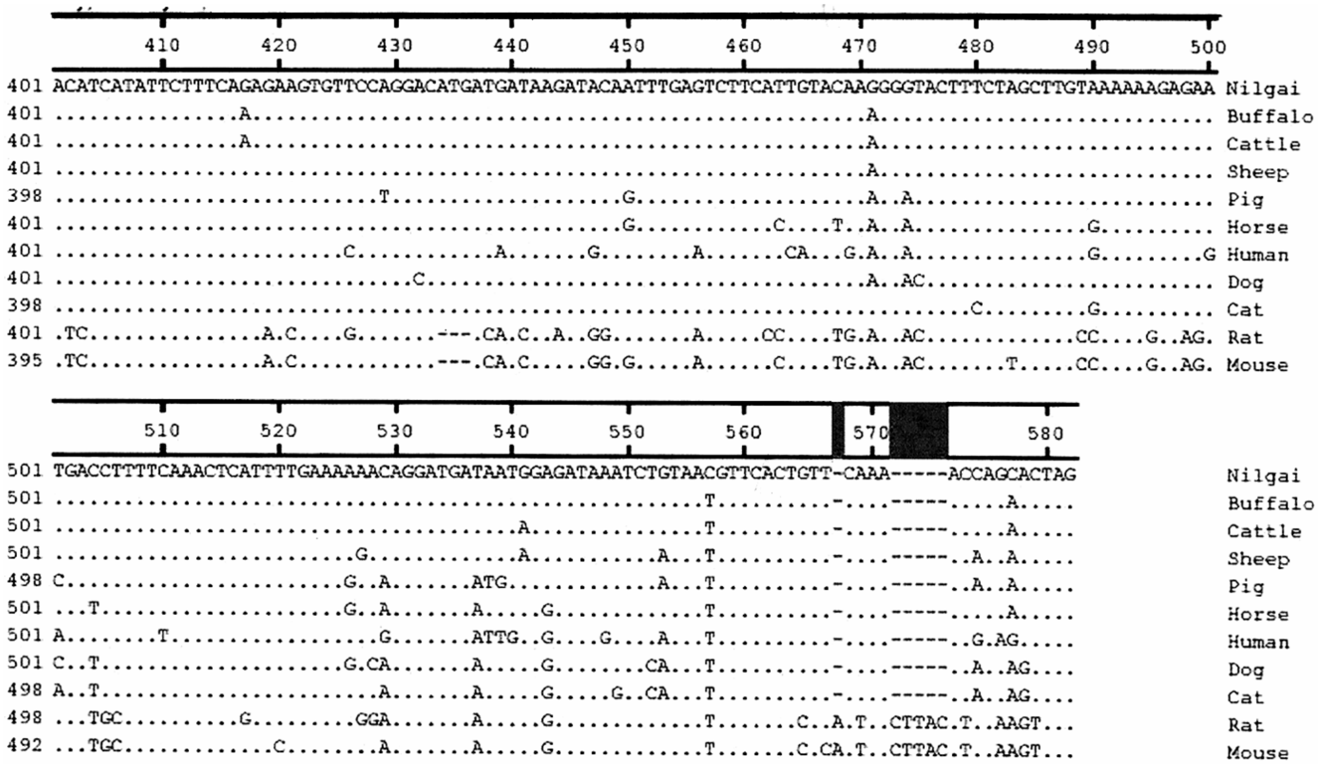


Fig. 1—Alignment of the nucleotide sequence of IL-18 cDNA from *Nilgai* (AY842499) with IL-18 sequences from buffalo, cattle, sheep, pig, horse, human, dog, cat, rat and mouse. Dots indicate nucleotide identity to the *Nilgai* sequence and dashes (-) represent gaps introduced to achieve best alignment.

Results and Discussion

The sequence analysis of *Nilgai* IL-18 revealed significant similarity with buffalo IL-18 sequence (98.5%). It had only seven nucleotides substitutions at positions 24, 25, 223, 285, 291, 417, 471, 557 and 577 but there were only two nucleotide substitutions in signal sequence at position 24 and 25 (Fig. 1). The nucleotide sequence of *Nilgai* IL-18 revealed 97.8% similarity with cattle, 97.6% with sheep, 91.0% with pig, 91.4% with horse, 85.6% with human, 88.0% with dog, 89.1% with cat, 68.4% with mouse and 68.4% with rat cDNA sequences. Analysis of gene sequence with human IL-18 revealed that it has five exons in the 582 bp ORF, namely exon 1 (79 bp), exon 2 (12 bp), exon 3 (135 bp) exon 4 (134 bp) and exon 5 (222 bp). There are four introns and all of them have the consensus sequence at both the 3´ and 5´ splice site. It follows the cis-splicing GU-AG rule, that is the consensus sequence required at the splice site has G and T as the first two residues and A and G as the last two residues[20]. The amino acid analysis has showed marked similarity with buffalo sequence (97.9%). It has only four amino acids substitution as compared to buffalo sequence at positions 9 (Y→N), 75 (S→P), 186 (M→T) and 193 (N→H) (Fig. 2). The predicted amino acid sequence of *Nilgai* IL-18 cDNA showed 96.9, 94.3, 78.2, 88.0, 87.0, 81.3 and 81.3% similarity with those of cattle, sheep, human, pig, horse, dog and cat, respectively.

IL-18 expression is regulated at both transcriptional and post-translational levels. There are two promoter sequences, which demonstrate both constitutive, and lipopolysaccharide (LPS) induced expression[21]. It is translated as an inactive pro-protein which has 193 amino acids polypeptide and its biologically active peptide is generated by cleavage of first 36 amino acids[22]. It was reported that IL-1β-converting enzyme (ICE) which selectively recognizes an Asp at the cleavage site, cleaved mouse Pro-IL-18 at an authentic processing site between Asp36 and Asn37[22]. Likewise, human[23] and cat[24] IL-18 was also shown to be cleaved by ICE between Asp36 and Tyr37. The amino acid sequence of *Nilgai* Il-18 cDNA reported here also contained an Asp36 and His37 residues corresponding to the cleavage site in ruminants (cattle, buffalo, sheep) IL-18 sequences. The cytokines of IL-1 family, including IL-18 have a characteristic IL-1 signature sequence, F-x(10)-F-x-

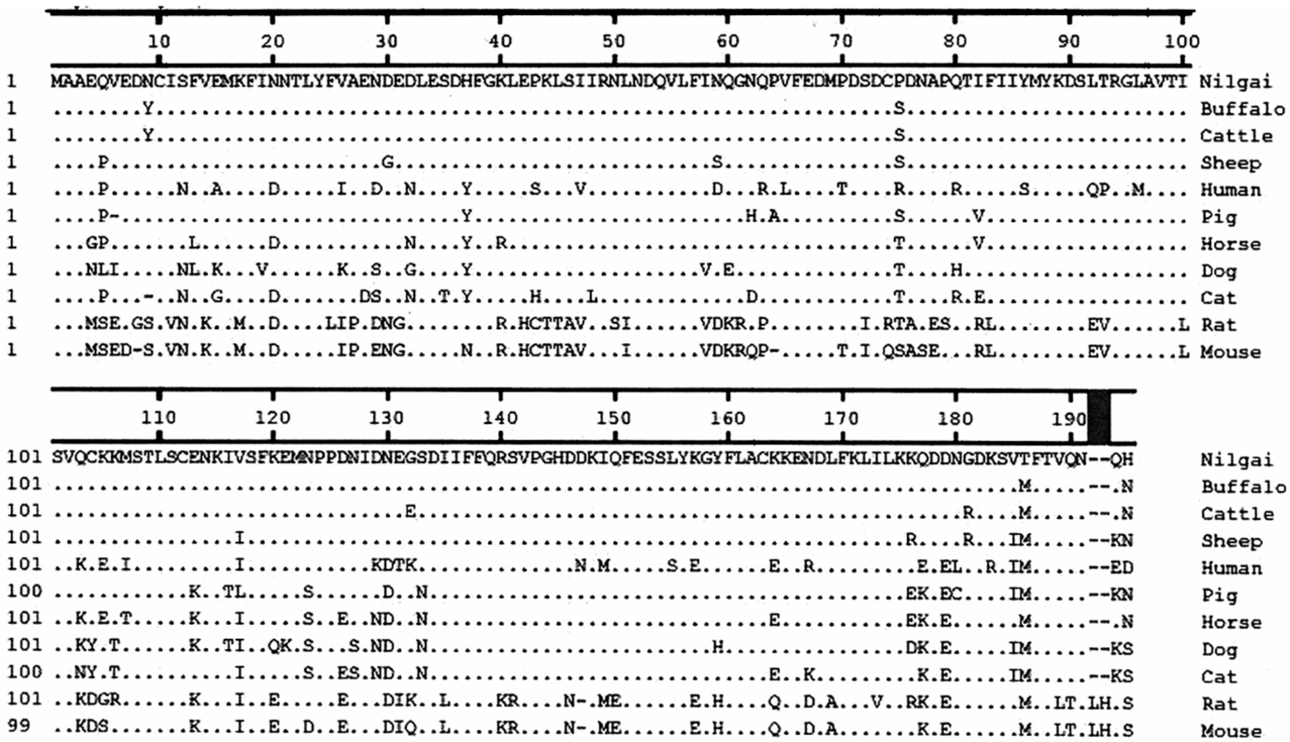


Fig. 2—Alignment of deduced amino acid sequence of the IL-18 gene of *Nilgai* with those of buffalo, cattle, sheep, human, pig, horse, dog, cat, rat and mouse. Dots indicate nucleotide identity to the *Nilgai* sequence and dashes (-) represent gaps introduced to achieve the best alignment.

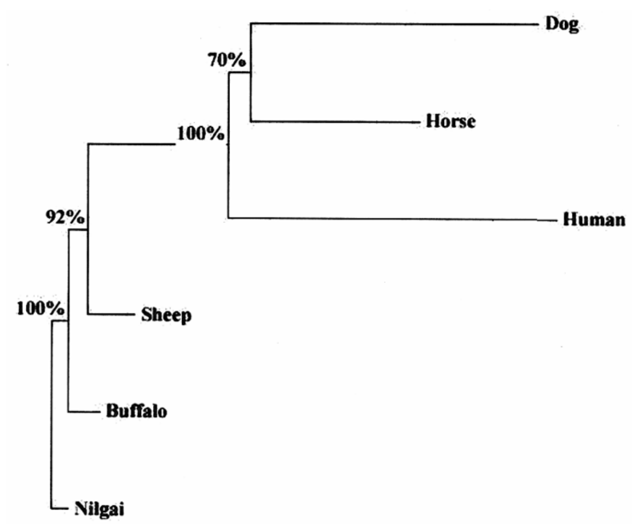


Fig. 3—Phylogenetic analysis computed from nucleotide sequences from IL-18 cDNA. The numbers on the tree indicate bootstrap values and the reliability of each branch was assessed using 1000 bootstrap replications.

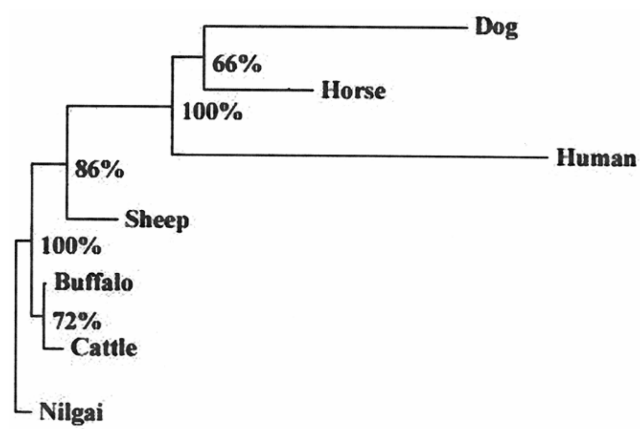


Fig. 4—Phylogenetic analysis is computed from amino acid sequences deduced from IL-18 cDNA. The numbers on the tree indicate bootstrap values.

S-[ALV]-x(2)-[AP]-x(2)-[FYLIV]-LIV]-x-T[14]. The predicted amino acid sequence of *Nilgai* IL-18 cDNA also included an IL-1 signature as shown in human and cat IL-18. From this finding, *Nilgai* Il-18 is expected to be processed and transported to the extracellular region in a manner similar to that of mouse and cat IL-18.

The phylogeny analysis of both nucleotide and deduced amino acid sequences showed that IL-18 sequences of various ruminant species form a single cluster. *Nilgai* IL-18 is evolutionarily more related to buffalo IL-18 sequence than to cattle sequence implying that they might have diverged recently from the same ancestor (Figs 3 & 4). The early branching of ruminant IL-18 indicated that both arose from a gene that exists before these lineages diverged.

Acknowledgement

Authors are grateful to Director, IVRI, Izatnagar for providing necessary facility to carry out this work and Central Zoo Authority, India for financial assistance.

References

1 Nakamura K, Okamura H, Nagata K, Komatsu T & Tamura T, Purification of α factor which provides α costimulatory signal for gamma interferon production, *Infect Immun*, 61 (1993) 64-70.

2 Okamura H, Tsutsui H, Kashiwamura S I, Yoshimoto T & Nakanishi K, Interleukin-18: α novel cytokine that augments both innate and acquired immunity, *Adv Immunol*, 70 (1998) 281-312.

3 Dinarello C A, IL-18: α Th 1-inducing, proinflammatory cytokine and new member of the IL-1 family, *J Allergy Clin Immunol*, 103 (1999) 11-24.

4 Takeuchi M, Nishizaki Y, Sano O, Ohata I, Ikeda M & Kurimoto M, Immunohistochemical and immuno-electron-microscopic detection of interferon-gamma-inducing facto (interleukin-18) in mouse intestinal epithelial cells, *Cell Tissue Res*, 289 (1997) 499-503.

5 Stoll S, Muller G, Kurimoto M, Saloga J, Tanimoto T *et al*, Production of IL-18 (IFN-gamma-inducing factor) messenger RNA and functional protein by murine keratinocytes, *J Immunol*, 159 (1997) 298-302.

6 Cameron L A, Taha R A, Tsicopoulos A, Kurimoto M, Olivernstein R *et al*, Airway epithelium expresses int4erleukin-18, *Eur Resp J,* 14 (1999) 553-559.

7 Elhofy A & Bost K L, Limited interleukin-18 response in *Salmonella* infected murine macrophages and in *Salmonella* infected mice, *Infect Immun*, 67 (1999) 5021-5026.

8 Olee T, Hashimoto S, Quach J & Lotz, M, IL-18 is produced by articular chondrocytes and induces proinflammatory and catabolic responses, *J Immunol*, 162 (1999) 1096-1100.

9 Foss D L, Zilliox M J & Murtaugh M P, Bacterially induced activation of interleukin-18 in porcine intestinal mucosa, *Vet Immunol Immunopathol*, 78 (2001) 263-277.

10 Kohno K, Kataoka J, Ohtsuki T, Suemoto Y, Okamoto I *et al*, IFN-gamma-inducing factor (IGIF) is a costimulatory factor on the activation of Th 1 but not Th 2 cells and exerts its effect independently of IL-12, *J Immunol*, 158 (1997) 1541-1550.

11 Robinson D, Shibuya K, Mui A, Zonin F, Murphy E *et al*, IGIF does not drive Th 1 development but synergizes with IL-12 for interferon-gamma production and activates iRAK and NF-kappa-β, *Immunity*, 7 (1997) 571-581.

12 Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kasiwamura S *et al*, IL-12 up-regulates IL-18 receptor expression on T cells Th 1 cells and B cells–synergism with IL-18 for IFN-g production, *J Immunol*, 161 (1998) 3400-3407.

13 Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A *et al*, Cloning of α new cytokine that induces IFN-gamma production by T cells, *Nature (Lond)*, 378 (1995) 88-91.

14 Ushio S, Namba M, Okura T, Hattori K, Nukada Y *et al*, Cloning of the cDNA for human IFN-gamma-inducing factor, expression in *Escherichia coli*, and studies of the biological activities of the protein, *J Immunol*, 156 (1996) 4274-4279.

15 Okano F, Satosh M, Ido T & Yamada K, Cloning of cDNA for cranine interleukin-18 and canine interleukin-1β, *J Interferon Cytokine Res*, 19 (1999) 27-32.

16 Argyle D J, McGillivery C, Nicolson L & Onions D E, Cloning sequencing and characterization of dog interleukin-18, *Immunogenetics*, 49 (1999) 541-543.

17 Shoda L K, Zarlenga D S, Hirano A & Brown W C, Cloning of α cDNA encoding bovine interleukin-18 and analysis of IL-18, *J Interferon Cytokine Res*, 19 (1999) 1169-1177.

18 Nicolson L, Penha-Goncalves M N, Keanie J L, Logan N A, Argyle D J & Onions D E, Cloning and sequencing of horse interleukin-12 and interleukin-18 cDNAs, *Immunogenetics*, 50 (1999) 94-97.

19 Schneider K, Puchler F, Baeuerle D, Elvers S, Staeheli P *et al*, cDNA cloning of biologically active chicken interleukin-18, *J Interferon Cytokine Res*, 20 (2000) 879-883.

20 Cristiano R J, Giordano S J & Steggles A W, The isolation and characterization of the bovine cytochrome b5 gene and a transcribed pseudogene, *Genomics*, 17 (1993) 348.

21 Kim J Y M, Kang H S, Paik S G, Pyun K H, Anderson K L *et al*, Roles of IFN consensus sequence binding protein and PU.1 in regulatinig IL-18 gene expression, *J Immunol*, 163 (1999) 2000-2007.

22 Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K *et al*, Activation of interferon-gamma inducing factor mediated by interleukin 1-β converting enzyme, *Science*, 275 (1997) 206-209.

23 Thornberry N A, Bull H G, Calaycay J R, Chapman K T, Howard A D *et al*, A Novel hetrodimeric cysteine protease is required for interleukin 1-β processing in monocytes, *Nature (Lond)*, 356 (1992) 768-774.

24 Ishizaka T, Setoguchi A, Masuda K, Ohno K & Tsujimoto H, Molecular cloning of feline interferon-γ-inducing factor (interleukin-18) and its expression in various tissues, *Vet Immunol Immunopathol*, 79 (2001) 209-218.