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

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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**: interleukin-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)². This cytokine has structural and functional similarities to IL-1 and IL-12, respectively²⁻³. 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 keratinocytes⁴⁻⁸. It is also expressed in Payer’s patches and in mesenteric lymph nodes⁹. 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 proliferation¹⁰⁻¹¹. IL-18 acts synergistically with IL-12 to induce IFN-γ secretion, in part by the upregulation of IL-18 receptors by IL-12¹². 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 mouse¹³, human¹⁴, dog¹⁵⁻¹⁶, horse¹⁸ and chicken¹⁹. 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.

**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% CO₂. 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’-ATGGTGCAAGAACAAGTAGA-3’, Reverse 5’-TAGTGCTGGTTTGTAGCAGTG-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 mM MgCl₂ 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/trecon).
Results and Discussion

The sequence analysis of Nilgai IL-18 revealed significant similarity with buffalo IL-18 sequence (98.5%). It had only seven nucleotide 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. 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. 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. 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. Likewise, human and cat 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.

S-\[ALV\]-x(2)-\[AP\]-x(2)-\[FYLIV\]-LIV-x-T

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.

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References