Cloning and Sequencing of a Bottle-Nosed Dolphin (Tursiops truncatus) Interleukin-1α and -1β Complementary DNAs

Yuuki INOUE, Takuya ITOU, Kenji UEDA1, Tatsuya OIKE2 and Takeo SAKAI
Department of Preventive Veterinary Medicine and Animal Health, Nihon University School of Veterinary Medicine, 1Department of Applied Biological Sciences, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252–8510 and 2Minamichita Beachland Aquarium, 428–1 Okuda Mihama, Chita, Aichi 470–3233, Japan
(Received 17 June 1999/Accepted 6 August 1999)

ABSTRACT. The bottle-nosed dolphin (Tursiops truncatus) IL-1α and IL-1β cDNA were cloned from mitogen stimulated peripheral blood mononuclear cells (PBMC) RNA utilizing the reverse transcription-polymerase chain reaction (RT-PCR). The sequences of these cDNAs showed that dolphin IL-1α and IL-1β clones contained open reading frames encoding 265 and 266 amino acids, respectively. Comparison of the deduced amino acid showed that dolphin IL-1α sequence shared 77, 77, 74, 69, 65, 64 and 63% similarity with the bovine, ovine, porcine, equine and feline, human and mouse IL-1α sequences, respectively. Similarly, the amino acid sequence showed that dolphin IL-1β shared 77, 77, 74, 69, 65, 64 and 63% similarity with the bovine, ovine, porcine, equine, feline, human and mouse IL-1β sequences, respectively. The relatedness of dolphin IL-1α and IL-1β were relatively distant with 21% amino acid homology.—KEY WORDS: bottle-nosed dolphin, IL-1α, IL-1β.

Interleukin-1 (IL-1) is a pluripotent cytokine produced mainly by monocytes, and many other types of cells, including fibroblasts, endothelial cells, dendritic cells and keratinocytes, may also produce one. Generally, IL-1 is produced by the stimulation, with a variety of substances, including endotoxin, phorbol esters, muramyl dipeptide and immune complexes. IL-1 plays an important role in inflammation, hematogenesis, and in immune, endocrine and cranial nerve system. IL-1 induces secretion of acute phase proteins, stimulates lymphocytes proliferation, lymphokine production and prostaglandin production, and enhances cellular infiltration through chemotaxis and mitogenicity [3, 5, 6]. Mature human IL-1α and IL-1β bind to the same receptor with similar affinity [4], exerting identical biological activity [20], though they share only 27% amino acid homology and have distinct isoelectric points. While bovine, ovine, porcine, equine, feline, human and mouse IL-1α genes have never been reported. The immune systems of terrestrial mammals such as human and mouse are well understood, while those of marine mammals, which encounter a wide variety of infectious agents in their environments are not. Little is known about cytokine genes in marine mammals with the exception of interleukin-2 (IL-2) [19], interleukin-4 (IL-4) [10], interleukin-6 (IL-6) [13] and interferon-γ (IFN-γ) [11]. In this study, the nucleotide and predicted amino acid sequences of the bottle-nosed dolphin IL-1α and IL-1β genes have been fully characterized [7, 17]. However, the cloning of marine mammal IL-1α and IL-1β genes have never been reported. The immune systems of terrestrial mammals such as human and mouse are well understood, while those of marine mammals, which encounter a wide variety of infectious agents in their environments are not. Little is known about cytokine genes in marine mammals with the exception of interleukin-2 (IL-2) [19], interleukin-4 (IL-4) [10], interleukin-6 (IL-6) [13] and interferon-γ (IFN-γ) [11]. In this study, the nucleotide and predicted amino acid sequences of the bottle-nosed dolphin IL-1α and IL-1β genes were described and compared with those of other species.

Peripheral blood samples were obtained with heparinized vacutainer tubes from the bottle-nosed dolphin (Tursiops truncatus) at the Minamichita Beachland Aquarium, Okuda, Aichi-ken, Japan.

The samples were isolated within 48 hr after blood collection. Ten ml of the blood sample was diluted with a four-fold volume of MEM containing 2% calf serum and 10 mM HEPES and layered onto 20 ml of Ficoll-paque (Amersham, Buckinghamshire, England). After centrifugation, peripheral blood mononuclear cells (PBMC) enriched fraction was isolated and washed twice with RPMI 1640 containing 10% fetal bovine serum, 3 mM L-glutamine, 25 μM 2-mercaptoethanol, 0.1 mM non-essential amino acids (GIBCO BRL, USA.), 1 mM pyruvic acid, 100 IU/ml penicillin, and 100 μg/ml streptomycin. PBMC were counted and resuspended in RPMI 1640 containing 7.5 μg/ml concanavalin-A (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) adjusted to 10⁶ cells/ml and then incubated in 24-well micro plates for 6 hr at 37°C before RNA extraction.

RNA was extracted from cell pellet by the ISOGEN (Nippon Gene, Toyama, Japan) according to the manufacturer’s instructions. cDNA was synthesized using oligo(dT)₁₆ primer (Perkin-Elmer, Foster City, CA) and RNA with Moloney-murine leukemia virus (MMLV) reverse transcriptase (Clontech, Palo Alto, CA) as described by Mullis and Faloona [18]. The primers to amplify IL1-α gene were: 5'-GCT CGA GTC AGC AAA GAA GTG AAG- 3' and 5'-GAG AGT AAA CAT TTA TTT AGA- 3'. The primers to amplify IL1-β gene were: 5'-CAG GTT TCT GAA ACA GCC ATG TCA TTT AGA ATT AC- 3'. The primers to amplify IL1-α gene were: 5'-GCT CGA GTC AGC AAA GAA GTG AAG- 3' and 5'-GAG AGT AAA CAT TTA TTT AGA- 3'. The primers to amplify IL1-β gene were: 5'-CAG GTT TCT GAA ACA GCC ATG TCA TTT AGA ATT AC- 3'. The primers to amplify IL1-α gene were: 5'-GCT CGA GTC AGC AAA GAA GTG AAG- 3' and 5'-GAG AGT AAA CAT TTA TTT AGA- 3'. The primers to amplify IL1-β gene were: 5'-CAG GTT TCT GAA ACA GCC ATG TCA TTT AGA ATT AC- 3'. The primers to amplify IL1-α gene were: 5'-GCT CGA GTC AGC AAA GAA GTG AAG- 3' and 5'-GAG AGT AAA CAT TTA TTT AGA- 3'. The primers to amplify IL1-β gene were: 5'-CAG GTT TCT GAA ACA GCC ATG TCA TTT AGA ATT AC- 3'.
cDNA showed that dolphin IL-1α similarity with that of bovine, ovine, porcine, equine, feline, human and mouse IL-1β, respectively. The sequence of cDNA showed that dolphin IL-1β clones contained open reading frames encoding 266 amino acids with a predicted molecular weight of 30,345 dalton. Based on the human, bovine, ovine and murine IL-1β sequences, the N-terminal amino acid of mature dolphin IL-1β might be Ala 114, giving a mature protein of 153 amino acids with a predicted molecular weight of 17,459 dalton.

The relatedness of IL-1α and IL-1β was relatively distant in dolphin with 21% amino acid homology, in comparison with that in bovine (23%), human (26%) and murine (22%) proteins. However, the IL-1α and IL-1β were indicated to bind to the same receptor causing the same biological functions [20]. The long N-terminal sequences did not appear to contain the usual signal sequences characterizing the secreted proteins. These leader sequences were predominantly hydrophilic in nature and could be proteolytically cleaved by proteases. The C-terminal region of dolphin IL-1α and IL-1β showed similar to each other in analysis of hydrophobicity/hydrophilicity plots (Hopp and Woods) [8], so that it would be able to bind to the IL-1 receptor as human IL-1.

The dolphin IL-1α and IL-1β gene sequences showed high similarity with the bovine and ovine sequences as well as those of the killer whale as in the case of IL-6 [13] and IL-2 [19] gene sequences. Moreover, the dolphin IL-4 [10] and IFN-γ [11] gene sequences are also similar to the bovine ones [15]. In the amino acid sequence level, therefore, cetacean cytokines tend to resemble bovine ones. The dolphin IL-1α and IL-1β appear to remain on the cells, associating with myristilation on specific Lys-82 and Lys 73 within the N-terminal amino acid as in human [22] (amino acid numbering based on the dolphin sequence). IL-1β converting enzyme cleaves the human IL-1β [14] at two sequence-related sites in the pro-protein. The dolphin IL-1β amino acid sequence also conserved cleavage sites, Asp-28—Gly-29 and Asp-19—Ala-120. The dolphin IL-1β has one potential N-linked glycosylation site, Asn-214—Thr-216, though human mature IL-1α and IL-1β are not known to be glycosylated. The dolphin mature IL-1β contains four cysteine residues (Cys-121, 137, 156, 184) and IL-1α has one (Cys-194), which would form an intrachain disulfide bridge. These seven amino acids (Arg-120, Leu-122, Phe-162, Ile-172, Lys-208, Lys218 and Glu-220) are clustered in the IL-1β molecule, forming a discontinuous binding site for the type I IL-1 receptor in human [23]. These amino acids were conserved in dolphin IL-1β (Leu-119, Phe-159, Ile-169, Lys215 and Glu-217). In human, two residues (Met-160 and Val-174) may be clustered in the IL-1α sequences, the N-terminal leader sequence as well as human and murine nucleotide sequences of IL-1α. Fig 2(a) compares the deduced amino acid sequence of the dolphin IL-1α protein with those of bovine, ovine, murine and human IL-1α. The dolphin IL-1α gene shared a sequence the bovine (88%), ovine (87%), porcine (87%), equine (85%), feline (83%), human (80%) and murine (67%) sequences homology. Similarly, the deduced amino acid sequence of dolphin IL-1α shared 77, 77, 74, 71, 65 and 57% similarity with that of bovine, ovine, porcine, equine, feline, human and mouse IL-1α, respectively. The sequence of cDNA showed that dolphin IL-1α clones contained open reading frames encoding 265 amino acids with a predicted molecular weight of 30,307 dalton. Based on the human, bovine, ovine and murine IL-1α sequences, the N-terminal amino acid of mature dolphin IL-1α might be Ser 113, giving a mature protein of 153 amino acids with a predicted molecular weight of 17,278 dalton.

Three IL-1β clones were isolated as was done in IL-1α gene cloning. The three clones had identical nucleotide sequences. The nucleotide sequence and deduced amino acid sequence are shown in Fig. 1(b). The sequence reported here is deposited in the Genbank date base [accession No. AB028216]. The dolphin IL-1β coding region consisted of 798 nucleotides, and the deduced 265 amino acids contained an IL-1α leader sequence as well as human and murine nucleotide sequences of IL-1α. The deduced amino acid sequence of the dolphin IL-1α protein shared 77, 77, 74, 67, 65 and 63% similarity with the bovine, ovine, porcine, equine, feline, human and mouse IL-1β, respectively. The sequence of cDNA showed that dolphin IL-1β clones contained open reading frames encoding 266 amino acids with a predicted molecular weight of 30,345 dalton. Based on the human, bovine, ovine and murine IL-1β sequences, the N-terminal amino acid of mature dolphin IL-1β might be Ala 114, giving a mature protein of 153 amino acids with a predicted molecular weight of 17,459 dalton.

The relatedness of IL-1α and IL-1β was relatively distant in dolphin with 21% amino acid homology, in comparison with that in bovine (23%), human (26%) and murine (22%) proteins. However, the IL-1α and IL-1β were indicated to bind to the same receptor causing the same biological functions [20]. The long N-terminal sequences did not appear to contain the usual signal sequences characterizing the secreted proteins. These leader sequences were predominantly hydrophilic in nature and could be proteolytically cleaved by proteases. The C-terminal region of dolphin IL-1α and IL-1β showed similar to each other in analysis of hydrophobicity/hydrophilicity plots (Hopp and Woods) [8], so that it would be able to bind to the IL-1 receptor as human IL-1.

The dolphin IL-1α and IL-1β gene sequences showed high similarity with the bovine and ovine sequences as well as those of the killer whale as in the case of IL-6 [13] and IL-2 [19] gene sequences. Moreover, the dolphin IL-4 [10] and IFN-γ [11] gene sequences are also similar to the bovine ones [15]. In the amino acid sequence level, therefore, cetacean cytokines tend to resemble bovine ones. The dolphin IL-1α and IL-1β appear to remain on the cells, associating with myristilation on specific Lys-82 and Lys 73 within the N-terminal amino acid as in human [22] (amino acid numbering based on the dolphin sequence). IL-1β converting enzyme cleaves the human IL-1β [14] at two sequence-related sites in the pro-protein. The dolphin IL-1β amino acid sequence also conserved cleavage sites, Asp-28—Gly-29 and Asp-19—Ala-120. The dolphin IL-1β has one potential N-linked glycosylation site, Asn-214—Thr-216, though human mature IL-1α and IL-1β are not known to be glycosylated. The dolphin mature IL-1β contains four cysteine residues (Cys-121, 137, 156, 184) and IL-1α has one (Cys-194), which would form an intrachain disulfide bridge. These seven amino acids (Arg-120, Leu-122, Phe-162, Ile-172, Lys-208, Lys218 and Glu-220) are clustered in the IL-1β molecule, forming a discontinuous binding site for the type I IL-1 receptor in human [23]. These amino acids were conserved in dolphin IL-1β (Leu-119, Phe-159, Ile-169, Lys215 and Glu-217). In human, two residues (Met-160 and Val-174) may be clustered in the IL-1α sequences, the N-terminal leader sequence as well as human and murine nucleotide sequences of IL-1α. Fig 2(a) compares the deduced amino acid sequence of the dolphin IL-1α protein with those of bovine, ovine, murine and human IL-1α. The dolphin IL-1α gene shared a sequence the bovine (88%), ovine (87%), porcine (87%), equine (85%), feline (79%), human (74%) and murine (75%) IL-1β genes. Similarly, the deduced amino acid sequence showed that dolphin IL-1β shared 77, 77, 74, 67, 65, 64 and 63% similarity with the bovine, ovine, porcine, equine, feline, human and mouse IL-1β, respectively. The sequence of cDNA showed that dolphin IL-1β clones contained open reading frames encoding 266 amino acids with a predicted molecular weight of 30,345 dalton. Based on the human, bovine, ovine and murine IL-1β sequences, the N-terminal amino acid of mature dolphin IL-1β might be Ala 114, giving a mature protein of 153 amino acids with a predicted molecular weight of 17,459 dalton.
CLONING AND SEQUENCING OF THE DOLPHIN IL-1

Fig. 1. Nucleotide sequence and deduced amino acid sequence of dolphin IL-1 \( \alpha \) (a) and IL-1 \( \beta \) (b) cDNAs.

(a) GCTCGAGTCGACAAAAAGATGAAG

25  ATGGCCCAAGTGCAGCCGACCTCTTGAGAACCTGAAGAATCTGTAAGAATGAAAAGAAG

187  85 TACAGTTCGGAATGACATGCTCTGTCGATGACGAGATGCTGATGACGCT

216  146 TACAGTTCGGAATGACATGCTCTGTCGATGACGAGATGCTGATGACGCT

285  245 CTTAGAGAAGCACATGATAGGATTTATACATCAATCCCTGAAACACTCT

354  314 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

423  382 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

492  451 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

560  519 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

628  587 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

696  655 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

725  684 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

794  753 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

823  782 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

852  811 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

881  840 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

910  869 AAGACAATGATAGGATTTATACATCAATCCCTGAAACACTCT

(b) CAGCTTCTCAGAAAAACGC

19  ATGGGAAAGCTGTAAGCCAAGCCGACACAGAAGTATGTTGGTCTAAGCATAGCAGAAGATGAC

52  93 CGCTTCTCAGAAAAACGC

182  123 CGCTTCTCAGAAAAACGC

211  152 CGCTTCTCAGAAAAACGC

299  240 CGCTTCTCAGAAAAACGC

338  279 CGCTTCTCAGAAAAACGC

427  368 CGCTTCTCAGAAAAACGC

515  456 CGCTTCTCAGAAAAACGC

604  545 CGCTTCTCAGAAAAACGC

692  633 CGCTTCTCAGAAAAACGC

781  722 CGCTTCTCAGAAAAACGC

870  811 CGCTTCTCAGAAAAACGC

Fig. 1. Nucleotide sequence and deduced amino acid sequence of dolphin IL-1 \( \alpha \) (a) and IL-1 \( \beta \) (b) cDNAs. Asterisk marks the stop codon. Underlined regions are a primer-binding site and, therefore, are not confirmed sequences.
Fig. 2. Alignment of dolphin, bovine, ovine, porcine, equine, feline, murine and human IL-1α (a) and IL-1β (b) amino acid sequences. The predicted N-terminal of the mature protein is marked with an asterisk. Those amino acid residues that are identical to the dolphin sequence are indicated by dashes. Where the amino acids are absent, it is denoted by a slash.
determine its biological activities. Particularly, IL1 is used as adjuvant [21]. Development of such adjuvant that uses it for the vaccination of dolphin may be able to prevent them from a wide variety of infectious agents. Though potential clinical applications of dolphin IL-1 as a therapeutic agent have never been investigated, recombinant dolphin IL-1 should be useful for advancement in prevention and control of dolphin diseases.

ACKNOWLEDGEMENT. This work was supported in part by grants from ‘High-tech Research Center Project’ of the Ministry of Education, Science, Sports and Culture.

REFERENCES