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SEQUENCE AND CHARACTERIZATION OF PHOCINE INTERLEUKIN 2

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ABSTRACT: To improve assessment of cellular immune responses in seals, northern elephant seal (*Mirounga angustirostris*) interleukin 2 (IL-2) has been characterized. The gene was cloned and sequenced from a 658 base pair (bp) cDNA generated from total RNA by reverse transcription-polymerase chain reaction (RT-PCR). The sequence encoded a 154 amino acid (aa) polypeptide that included a 20 aa putative signal peptide. Seal IL-2 was found to share considerable identity with published sequences. Nucleotide sequence analysis of phocine (seal) IL-2 with canine, feline, human, trichechine (manatee), bovine and murine sequences demonstrated 93, 92, 86, 82, 78 and 71% identity, respectively. Analysis of the derived amino acid sequences demonstrated 88, 89, 78, 71, 66 and 60% identity, respectively. Interleukin-2 sequence identities appear to reflect evolutionary proximity among the analyzed species, and importantly, those residues identified as critical to IL-2 biological activity and receptor binding are largely conserved. To examine the kinetics of IL-2 mRNA expression, northern elephant seal lymphocytes were stimulated with the mitogen concanavalin A (Con A), and RNA was collected at several time points thereafter. The RT-PCR demonstrated that seal IL-2 mRNA expression peaks in the first 8 hr following Con A stimulation. Lastly, genomic DNA from northern elephant seal, harbour seal (*Phoca vitulina*) and California sea lion (*Zalophus californianus*) was used as template to identify and clone genomic IL-2. Partial sequence of the genomic clones demonstrated nearly complete identity among the three species. Sequence identity indicates that probes constructed from the northern elephant seal IL-2 gene will be effective in assessing IL-2 in other pinniped species.

Key words: Cellular immune response, interleukin 2, *Mirounga angustirostris*, northern elephant seal, nucleotide sequence analysis, phocids, reverse transcription-polymerase chain reaction, cross-species reactivity, cytokine mRNA expression.

INTRODUCTION

The field of marine mammal immunology has expanded in recent years due in part to widespread interest generated by epidemic events that caused mass mortalities in local or regional marine mammal populations. The most dramatic recent event occurred in northern Europe where in 1988–89 over 17,000 harbour seal deaths (*Phoca vitulina*) were attributed to distemper-like symptoms (Osterhaus et al., 1988). Several other distemper-like outbreaks were reported, including one that claimed thousands of Baikal seals (*Phoca sibirica*), from a population endemic to Lake Baikal in Siberia (Grachev et al., 1989), and one that claimed hundreds of striped dolphins (*Stenella coeruleoalba*) in the Mediterranean (Van Bresse et al., 1991). The causative agents in these events

were identified as viral, but there was considerable debate on why such a large number of animals succumbed to disease. The epidemics exposed how poorly immune responses in marine mammals are understood and have provided impetus for the expansion of basic knowledge in marine mammal immunology.

Immunological assays have been adapted from human or laboratory animal systems for marine mammals (De Swart et al., 1993; King et al., 1993; Ross et al., 1993; DiMolfetto-Landon et al., 1995; Erickson et al., 1995), and reagents have been developed specifically for marine mammal species (Romano et al., 1992; Cashman et al., 1996; King et al., 1996). Since species differences can result in altered biological activity, the development of species-specific reagents often is critical

to success. To facilitate the ability to evaluate lymphocyte responses in northern elephant seals (*Mirounga angustirostris*), species-specific interleukin 2 (IL-2) has been cloned, sequenced, and partially characterized.

Interleukin 2 is an inducible cytokine produced by activated T cells in response to antigenic or mitogenic stimulation. It was originally termed T cell growth factor and is an autocrine growth factor that is produced and secreted in association with production surface expression of the IL-2 receptor (IL-2R) (Taniguchi and Minami, 1993). Interleukin 2 binding to IL-2R on the T cell surface initiates a signal cascade that results in cell maturation and proliferation. The expanded population of activated T cells can then mount an immune response against pathogens by facilitating an antibody response, by stimulating other cytotoxic immune cells, or by actively killing infected cells. We chose to characterize IL-2 because it is critical to immune responses against intracellular pathogens and because it is known to be partially species restricted (English et al., 1985; Fenwick et al., 1988). Multiple sequence alignment of published IL-2 sequences indicated that IL-2 is well conserved among widely divergent species. Other work in our laboratories suggested that conserved regions would be useful in isolating northern elephant seal IL-2 (Hash et al., 1994). Once cloned and sequenced, northern elephant seal IL-2 could be used as a probe to assess IL-2 production by lymphocytes. It also was deemed likely that a northern elephant seal probe would be applicable to related species. Northern elephant seals are members of the suborder Pinnipedia which includes 19 seal species in the family Phocidae, nine fur seal and sea lion species in the family Otariidae, and one walrus species in the family Odobenidae (Reeves et al., 1992). Herein we report the sequence, kinetics of mRNA expression, and cross-species reactivity for northern elephant seal IL-2.

MATERIALS AND METHODS

Whole blood was collected from a northern elephant seal pup at The Marine Mammal Center (TMMC, Sausalito, California, USA) into heparin vacutainer tubes and shipped overnight to our laboratories at Texas A&M University (College Station, Texas, USA). Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation over Ficoll-hypaque 1077 (Sigma Chemical Company, St. Louis, Missouri, USA). Specifically, vacutainer tubes were centrifuged at $850 \times g$ for 15 min at 10 C. The buffy coats were collected into sterile polypropylene tubes, diluted with an equal volume of Alsever's solution and overlaid on one volume Ficoll-hypaque 1077 (Goddeeris et al., 1986). Following gradient centrifugation at $850 \times g$ for 45 min at 18 C and PBMC collection, PBMC were washed in Alsever's solution at $500 \times g$ for 15 min at 10 C and subsequently at $250 \times g$ for 10 min at 10 C. The PBMC were cultured in RPMI 1640 (Gibco BRL, Grand Island, New York, USA) supplemented with 10% heat-inactivated fetal calf serum (HIFCS, Hyclone Laboratories, Logan, Utah, USA), 50 mM 2-mercaptoethanol (Sigma Chemical Company), 2 mM L-glutamine (Gibco BRL), and 50 $\mu\text{g/ml}$ gentamicin (Gibco BRL), hereafter referred to as complete RPMI (cRPMI), with 2 $\mu\text{g/ml}$ concanavalin A (Con A, Sigma Chemical Company) in 24-well plates at 2×10^6 cells/well for 3 days at 37 C in a humidified atmosphere of 5% CO_2 in air. Cells were then harvested, washed, and restimulated with 1 $\mu\text{g/ml}$ Con A for 8 hr. At 8 hr, they were harvested for total RNA extraction in RNazol B according to the manufacturer's instructions (Biotecz Inc., Houston, Texas, USA).

Elephant seal IL-2 was cloned and sequenced essentially as described (Hash et al., 1994). Primers to screen for northern elephant seal IL-2 were selected from consensus regions identified with MacVector DNA analysis software (IBI, New Haven, Connecticut, USA) from human, murine, bovine, porcine, and feline IL-2 sequences. Within consensus regions, the feline sequence (GenBank Sequence Database accession no. L19402, National Center for Biotechnology Medicine, Bethesda, Maryland, USA; Cozzi et al., 1993) was found to differ slightly from other species. Given the evolutionary proximity of felids and phocids within the order Carnivora, feline-specific primers were selected and synthesized (Gene Technologies Laboratory, Texas A&M University, College Station, Texas, USA). First strand cDNA synthesis was performed on 0.5 μg total RNA in a Perkin Elmer Cetus thermal cycler (Perkin

Elmer Corporation, Branchburg, New Jersey, USA) using MuLV reverse transcriptase and 50 pmol of the feline IL-2 reverse primer, 5'-GCACTTCCTCCAGAGGTTTC (nucleotides [nt] 290–309). The reaction mix was incubated at 42 C for 15 min, followed by 99 C for 5 min, and 5 C for 5 min. Fifty pmol of the feline IL-2 forward primer, 5'-CTCACAGTAACCTCAACTCC (nt 12–31), was added, and the reaction mix was adjusted for PCR conditions, including the addition of *Amplitaq* DNA polymerase (Perkin Elmer Corporation). The reaction mix was initially heated to 94 C for 2 min, then amplified for 35 cycles under the following conditions: 95 C 1 min; 40 C 1 min, 72 C 2 min, with a 5 sec autoextension. A 5' primer internal to the original (i.e., a nested primer) was used to obtain a partial cDNA for elephant seal IL-2. The nested reaction was conducted using the feline IL-2 forward primer 5'-CAGTAACCTCAACTCCTGCC (nt 16–35) (Gene Technologies Laboratory) and the original reverse primer. The reaction yielded a discrete PCR product that approximated the expected 293 bp size and was cloned into pCRII using the TA Cloning Kit as specified by the manufacturer (Invitrogen Corporation, San Diego, California, USA). Independent clones were sequenced by the chain termination method, using the Sequenase 7-deaza-dGTP Sequencing Kit, as specified by the manufacturer (USB, Cleveland, Ohio, USA). Elephant seal-specific primers were selected from the resulting sequence and were used in an RT-PCR reaction to synthesize a 452 bp cDNA that included the missing portion of the gene. The second fragment was cloned and sequenced as described above. To control for errors introduced during PCR DNA synthesis, several clones were generated for each fragment and were sequenced along both strands using appropriate overlapping primers. The two IL-2 cDNA fragments had an overlap region of 86 bp. A complete cDNA for the IL-2 gene was generated by conducting PCR on plasmid DNA from the two fragments using the outermost forward and reverse primers. Northern elephant seal IL-2 sequence (GenBank accession no. U79187) was analyzed against GenBank sequences using Genetics Computing Group (GCG) software (Wisconsin Sequence Analysis Package, Madison, Wisconsin, USA).

For the kinetics experiment, 30 ml of whole blood was collected in acid citrate dextrose (ACD) vacutainer tubes from a northern elephant seal pup at the Marine Mammal Care Center in Fort MacArthur (San Pedro, California, USA). The PBMC, isolated as described above in the Sea World of California Animal Care Laboratory, were cryopreserved in

HIFCS (Hyclone Laboratories) containing 10% dimethylsulfoxide (DMSO). Cells were frozen at a rate of 1 C per min to –80 C then stored in the liquid nitrogen vapor phase. Cryopreserved cells were thawed in a 37 C water bath and diluted in cRPMI to a final volume of 10 ml. Cells were washed and plated in 24-well plates at 2×10^6 cells per well with 2 μ g/ml Con A. At 4, 8, 12, 16, 20, and 24 hr post-stimulation, total RNA was harvested in TRIzol Reagent (Gibco BRL) and isolated according to the manufacturer's instructions. RNA integrity was verified by size fractionation on a 1% agarose gel stained with ethidium bromide (Rodriguez et al., 1996).

The kinetics of IL-2 mRNA expression were evaluated by the method of semi-quantitative RT-PCR, essentially as described by Chitko-McKown et al. (1995). First strand cDNA synthesis was performed with total RNA using oligo d(T) under the following conditions: 25 C 10 min, 42 C 15 min, 99 C 5 min in a Perkin Elmer 2400 thermal cycler. Elephant seal IL-2 mRNA was detected by PCR using species-specific forward primer 5'-GCATCGCAC-TATCTCTTGAC (nt 49–69) (Gibco BRL) and reverse primer 5'-CAAGTCAGTGTTGA-GAAGATGC (nt 466–487) (Gibco BRL) on cDNA generated from 0.25 μ g of total RNA. Reaction mixtures were incubated at 94 C for 30 sec, followed by an 80 C incubation for 2 min and 30 sec during which *Amplitaq* DNA polymerase was added. Reactions were then amplified under the following conditions: 95 C 15 sec, 68 C 30 sec, 72 C 1 min, with a 2 sec auto extension. The number of cycles used to amplify IL-2 was optimized by conducting PCR reactions at 25, 30, 35, 40, and 45 cycles and subjecting the products to electrophoretic densitometry analysis by the IS1000 Digital Imaging System (Alpha Innotech Corporation, San Leandro, California, USA). Thirty five cycles were determined optimal for amplification of IL-2. Actin was amplified from each cDNA sample to control for disparity in the actual amount of RNA present during the RT reaction. Elephant seal actin mRNA was detected by PCR using bovine β -actin-specific primers (Gibco BRL) on cDNA from 0.125 μ g of total RNA template. Forward β -actin primer, 5'-CCTTTTACAACGAGCTGCGTGTC (nt 38–60), and reverse primer, 5'-ACGTAGCAGAGCTTCTCCTTGATG (nt 405–428), were used to produce a 391 bp product. The PCR conditions were as described above apart from the use of 60 C annealing temperature. Actin PCR reactions were conducted at 15, 20, 25, 30, and 35 cycles, and 25 cycles were determined to be optimal by densitometry analysis. The PCR products from the Con A stimulation

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-23 cagtaacctc aactcctgac acaatgtgca aaatgcaact cttgtcttgc atgcactat 37
      M C K M Q L L S C I A L S
38 ctcttgtaact tgtcgcaaac agtgcaccta ctacttcgtc tacgaaggaa acacagcaac 97
      L V L V A N S A P T T S S T K E T Q Q Q
98 agttggagca attgctgctg gatttacggt tgccttgaa tggagttaat aattatgagg 157
      L E Q L L L D L R L L L N G V N N Y E D
158 accctaaact ctccaggatg ctcacattta aattctacac gcccaagaag gccacagaat 217
      P K L S R M L T F K F Y T P K K A T E L
218 tgacacatct tcaatgtcta gcagaagaac tcaaacctct ggaggaagtg ctatatattag 277
      T H L Q C L A E E L K P L E E V L Y L A
278 ctaaaagcaa aaactttcac ttgacagaca tcaaggaatt aatgagcaat atcaatgtaa 337
      Q S K N F H L T D I K E L M S N I N V T
338 cactttctgaa actaaaggga tctgaacaa gattcaaatg tgaatatgat gacgagacag 397
      L L K L K G S E T R F K C E Y D D E T A
398 caaccattac agaatttctg aacaaatgga ttactttttg tcaaagcatc ttctcaacac 457
      T I T E F L N K W I T F C Q S I F S T L
458 tgacttgata attgacttga taattgtctc ctatttataa tataccaggc tatttattga 517
      T *
518 aatattataa attcatattt attttttgac atatggtttg ctaccttttg taattatatt 577

578 cttatacttc atatgataaa tatggatctt ttaagattct ttttgtaagc cctagggcgtg 637

638 ctaaaaactcac

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FIGURE 1. The cDNA nucleotide sequence from northern elephant seal IL-2 and deduced amino acid sequence. Outermost forward and reverse primers used in PCR reactions to generate the complete IL-2 cDNA are underlined. The initiator methionine codon is double underlined, and the putative 20 aa signal peptide is shown in italics.

time course were electrophoresed on a 1% agarose gel stained with ethidium bromide. Densitometry analysis of the products was conducted to semi-quantitate the kinetics of IL-2 mRNA expression. The results were standardized against densitometric analysis of β -actin products. Controls included a positive plasmid control, a negative no-template control, and negative no-RT controls.

To evaluate cross-species reactivity, whole blood collected from elephant seal, harbour seal, and California sea lion (*Zalophus californianus*) pups by TMMC was used as a source of genomic DNA. After centrifuging vacutainer tubes at $900 \times g$ at 18 C for 15 min, buffy coats were collected and lysed with an equal volume of TE buffer, pH 7.5 (10 mM Tris, 1 mM EDTA) containing 1% sodium dodecyl sulfate (SDS) at 37 C for 30 min. The suspension was treated with 50 μ g/ml RNase A at room temperature for 1 hr, followed by 50 μ g/ml proteinase K at 50 C for 2 hr. Genomic DNA was extracted with an equal volume of buffer-saturated phenol:chloroform, then chloroform alone and was purified by dialysis. Samples were concentrated using Centrprep-10 concentrator tubes as specified by the manufacturer (Amicon, Beverly, Massachusetts, USA). The PCR was conducted using elephant seal-specific primers, forward primer 5'-GCA-TCGCACTATCTCTTGAC (nt 49–69) and reverse primer, 5'-CTGTAATGGTTGCTGTCTC GTC (nt 411–432), with 100 ng genomic DNA as template. The reactions, using Elongase enzyme mix according to the manufacturer's in-

structions (Gibco BRL), were amplified for 30 cycles at 95 C 30 sec, 52 C 30 sec, and 68 C for 4 min in a Perkin Elmer 2400 thermal cycler. The PCR products were found to approximate the 3.8 kb expected size. They were ligated into pCRII and cloned as described above. To prepare DNA for automated sequencing on an ABI Prism 373 Genetic Analyzer (Perkin Elmer Corporation), DNA synthesis was conducted on plasmid DNA with elephant seal-specific IL-2 primers and the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit, as specified by the manufacturer (Perkin Elmer Corporation). Genomic IL-2 was sequenced in both directions over a 350 bp region. The region overlapped an exon-intron junction, and the exon sequence, comprising 90 bp was analyzed using GCG software.

RESULTS

The PCR primers selected from conserved regions of the feline IL-2 sequence (GenBank accession no. L19402; Cozzi et al., 1993) were applied in RT-PCR reactions to northern elephant seal RNA template and produced a 293 bp fragment and a 452 bp fragment for elephant seal IL-2. The two fragments had a region of overlap spanning 86 bp and together defined a 658 bp cDNA of the IL-2 gene (Fig. 1). The sequence had an open reading frame for a precursor polypeptide of 154 amino acids,

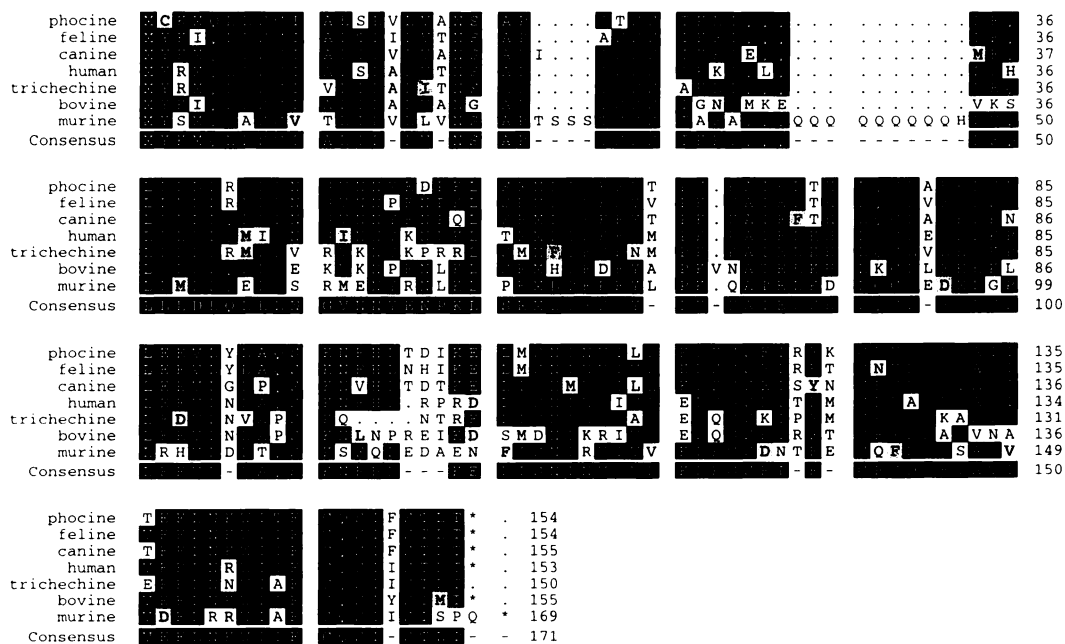


FIGURE 2. GCG multiple sequence alignment of the deduced amino acid sequence for phocine IL-2 against canine (GenBank accession no. D30710; Dunham et al., 1995), feline (L19402; Cozzi et al., 1993), human (X01586; Devos et al., 1983), bovine (M13204, M17428; Reeves et al., 1986), trichechine (U09420; Cashman et al., 1996), and murine (K02797; Kashima et al., 1985). Conserved amino acids are blocked in a black background, non-conserved amino acids are displayed in white. The consensus sequence is displayed along the bottom of the alignment.

starting with an initiator methionine codon at nt 1 and ending with a TGA termination codon (nt 462–464). Based on comparison with published IL-2 sequences (Devos et al., 1983; Reeves et al., 1986; Goodall et al., 1991; Dunham et al., 1995), a putative 20 amino acid (aa) signal peptide precedes the 134 aa mature peptide.

The elephant seal nucleotide sequence demonstrated extensive identity with published sequences for canine (Dunham et al., 1995), feline (Cozzi et al., 1993), human (Devos et al., 1983), trichechine (Cashman et al., 1996), bovine (Reeves et al., 1986) and murine IL-2 (Kashima et al., 1985). Identity was highest with canids and felids (93 and 92%, respectively) and dropped off somewhat in the other comparisons 86, 82, 78 and 71%, respectively. Further, identity among the predicted amino acid sequences was consistent with a high degree of conservation in the IL-2 gene (Fig. 2). Again, canine and feline

demonstrated the closest relationship with elephant seal (88 and 89%, respectively), and reduced identity was observed with other comparisons (78, 71, 66 and 60%, respectively).

To improve efficiency in assaying for IL-2 mRNA expression, we investigated the kinetics of expression from Con A-stimulated seal lymphocytes. Elephant seal IL-2 and bovine β -actin primers were used to conduct RT-PCR reactions on total RNA collected from PBMC at 4, 8, 12, 16, 20, and 24 hr post-stimulation. Control reactions performed in the absence of RT did not contain PCR products (data not shown). RT-PCR products were visualized on agarose gels (Fig. 3) and subjected to densitometry analysis (Fig. 4). IL-2 densitometry measurements were then standardized against analogous actin densitometry measurements (Degen et al., 1983). The results demonstrate that elephant seal IL-2 mRNA was detectable at all time

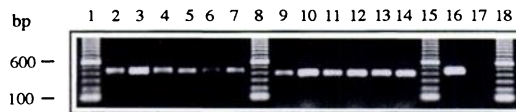


FIGURE 3. Semi-quantitative RT-PCR for IL-2 mRNA expression showing PCR products electrophoresed on a 1% agarose gel. Lanes 1, 8, 15, and 18 contain a 100 bp ladder (Gibco BRL); lanes 2–7 illustrate IL-2 cDNA at 4, 8, 12, 16, 20, and 24 hr after Con A stimulation; lanes 9–14 illustrate β -actin at the same time points; lane 16 contains a plasmid positive control; lane 17 contains a no template negative control.

points but peaked in the first 8 hr following mitogen activation and declined thereafter.

To validate the assumption that probes derived from northern elephant seal IL-2 sequence would be suitable for use with other pinniped species, we conducted a limited analysis of IL-2 in another phocid species, harbour seal, and in an otariid, California sea lion. Genomic DNA from harbour seal, California sea lion and northern elephant seal was isolated, subjected to PCR for IL-2, cloned and sequenced. The sequenced exon region corresponded to elephant seal cDNA nt 48–146. As expected, IL-2 in harbour seal and in California sea lion closely matched that of elephant seal. Multiple sequence analysis of the nucleotide sequences revealed only two coding differences in this region (Fig. 5). While these data cannot be assumed to be representative of homology between the species, the near identity indicates that a probe chosen from the conserved sequence in this region would be able to identify IL-2 in these species and likely in all other members of the families Phocidae and Otariidae.

DISCUSSION

Elephant seal IL-2 was identified as a 154 aa polypeptide containing a 20 aa putative signal peptide. Comparison with other IL-2 sequences indicated that there was a high degree of conservation among widely divergent species. The greatest similarities were observed among phocine, ca-

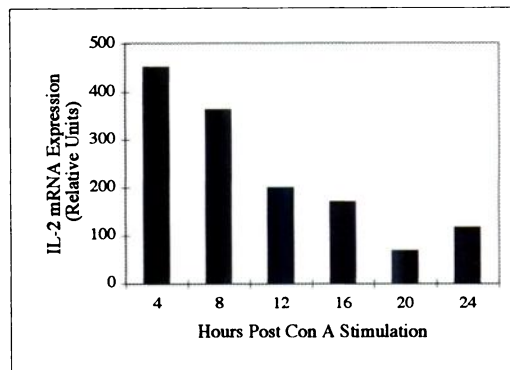


FIGURE 4. Semi-quantitative RT-PCR for IL-2 mRNA expression illustrating densitometry analysis of IL-2 mRNA time course, where IL-2 measurements have been standardized against β -actin.

nine, and feline IL-2, which is consistent with their evolutionary proximity as members of the order Carnivora.

Human and murine IL-2 have been exhaustively studied to identify those residues that are most critical to biological activity. It is known that the native protein has three cysteine residues at positions -58, -105, and -125 in the human homologue. The first two form an intramolecular disulfide bridge, while C-125 is a free sulfhydryl group (Robb et al., 1984). Site-specific mutations and deletions of these residues have shown that loss or inappropriate formation of the intramolecular disulfide bridge leads to loss of biological activity (Wang et al., 1984). Although loss of the third cysteine compromised biologic activity, non-conservative substitutions did not, suggesting that the third cysteine is only important in maintaining the structural requirements of that region (Liang et al., 1988). Not surprisingly, all three of these cysteine residues were conserved in elephant seal IL-2. Glycosylation sites have also been noted in IL-2 sequences. For example, the bovine IL-2 sequence has a potential N-glycosylation site (Asn-X-Ser/Thr) at Asn-70 (Cerretti et al., 1986). This site also is present in other ruminants (i.e. deer, GenBank accession no. U14682, goat X76063, sheep X55641; Seow et al., 1990) but is absent from non-

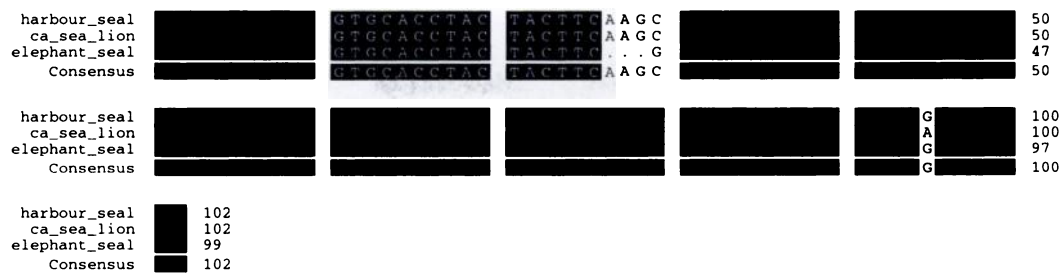


FIGURE 5. GCG multiple sequence alignment of nucleotide sequence from genomic IL-2 of elephant seal, harbour seal, and California sea lion. Conserved residues are blocked in black; non-conserved are unblocked.

ruminant species, including seal. In seal IL-2, a potential N-glycosylation site was noted at Asn-111; this site is conserved in other carnivores (i.e., cat, dog D30710; Dunham et al., 1995) and in the pig (X56750; Goodall et al., 1991).

Human IL-2 is composed of four helices and one sheet (Mott et al., 1992). It binds its homologue receptor which is composed of three interacting subunits α , β , γ (Minami et al., 1993). The high affinity IL-2R that elicits a full biological response is an $\alpha\beta\gamma$ heterotrimer. Lesser affinity binding can occur between IL-2 and $\alpha\beta$ and $\beta\gamma$ heterodimers and with α or β subunits singly. In the mouse, 21 residues have been identified as structurally critical; 19 have been implicated in binding to IL-2R α , three residues for β and one for γ (Zurawski et al., 1993). When these residues are compared with analog seal residues, a high degree of conservation is observed (Table 1). Nineteen of the 23 residues presumably involved in binding IL-2R subunits are conserved. Three of the differences occur at α -binding residues, N⁴⁷ \rightarrow D³³, D⁷⁵ \rightarrow E⁶¹ and V¹³⁰ \rightarrow T¹¹⁶. The fourth occurs at one of three identified β -binding residues, N⁹⁹ \rightarrow E⁸⁵. Seventeen of 21 residues involved in structural integrity are conserved. Three of the four substitutions maintain the hydrophobic nature of the site. The last substitution, F¹²² \rightarrow Y¹⁰⁸ substitutes a hydrophilic residue for a hydrophobic one but maintains the phenyl side chain. Where conservation between the complete seal and mouse IL-2 genes is relatively low (60%, see Fig. 2), conservation

among the purported critical residues is 82% which is consistent with the importance of these residues to biological activity.

Sequence analysis of genomic IL-2 from harbour seal, California sea lion and northern elephant seal suggested that elephant seal sequence can be used to assess IL-2 in other phocids and in otariids. There are several means by which cellular immune responses in pinnipeds may be assessed. Several studies have measured the proliferative responses of seal lymphocytes to mitogen or antigen (DeSwart et al., 1993; Ross et al., 1993). DiMolfetto-Landon et al. (1993) reported the development of an IL-2R assay. The addition of an IL-2 mRNA assay provides another analysis parameter that is unique but complementary to the existing tests. The next step would be to subclone northern elephant seal IL-2 into an expression system. Recombinant northern elephant seal IL-2 would be expected to significantly enhance research efforts with seal T lymphocytes by providing a homologous growth factor for *in vitro* cell culture. Cytokine reagents are critical to characterizing T lymphocyte responses to specific antigens which is the first step towards defining and manipulating pathogen-specific protective immunity in exotic animal species.

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TABLE 1. Comparison of significant residues from the mature peptide of mouse (m) IL-2 against seal (s) IL-2.

mIL-2 ^a	sIL-2	Function
N ⁴⁴	N ³⁰	α ^b
N ⁴⁷	D ³³	α ^d
K ⁴⁹	K ³⁵	α
R ⁵²	R ³⁸	α
M ⁵³	M ³⁹	α
L ⁵⁴	L ⁴⁰	α
F ⁵⁶	F ⁴²	α
K ⁵⁷	K ⁴³	α
F ⁵⁸	F ⁴⁴	α
Y ⁵⁹	Y ⁴⁵	α
D ⁷⁵	E ⁶¹	α ^d
E ⁷⁶	E ⁶²	α
P ⁷⁹	P ⁶⁵	α
V ⁸³	V ⁶⁹	α
L ⁸⁶	L ⁷²	α
Q ⁸⁸	Q ⁷⁴	α
D ¹²⁴	D ¹¹⁰	α
T ¹²⁸	T ¹¹⁴	α
V ¹³⁰	T ¹¹⁶	α ^d
D ³⁴	D ²⁰	β ^e
N ⁹⁹	E ⁸⁵	β ^d
N ¹⁰³	N ⁸⁹	β
Q ¹⁴¹	Q ¹²⁷	γ ^g
L ²⁸	L ¹⁴	H ^c
L ³⁵	L ²¹	H
L ³⁸	L ²⁴	H
M ⁴²	V ²⁸	H ^d
P ⁶¹	P ⁴⁷	H
A ⁶⁴	A ⁵⁰	H
L ⁷⁰	L ⁵⁶	H
L ⁷³	L ⁵⁹	H
L ⁷⁷	L ⁶³	H
L ⁸⁰	L ⁶⁶	H
L ⁸⁴	L ⁷⁰	H
I ¹⁰¹	M ⁸⁷	H ^d
I ¹⁰⁴	I ⁹⁰	H
V ¹⁰⁸	L ⁹⁴	H ^d
L ¹¹¹	L ⁹⁷	H
F ¹²²	Y ¹⁰⁸	H ^d
F ¹³²	F ¹¹⁸	H
L ¹³³	L ¹¹⁹	H
W ¹³⁶	W ¹²²	H
C ⁷²	C ⁵⁸	C ^f
C ¹²⁰	C ¹⁰⁶	C

^a From Zurawski et al. (1993).^b α indicates residues associated with binding to IL-2R α subunit.^c H indicates a hydrophobic residue presumed to be of structural importance.^d Indicates a non-conserved residue.^e β indicates residues associated with binding to IL-2R β subunit.^f C indicates a cysteine residue that takes part in the formation of a disulfide bridge.^g γ indicates the residue associated with binding to IL-2R γ subunit.

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