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Recent Cytokine Findings and Implications Toward Health Assessment of the Bottlenose Dolphin (*Tursiops truncatus*)

Anna Vecchione,[1] Margie M. Peden-Adams,[2, 3, 4]
Tracy A. Romano,[4] and Patricia A. Fair[5]

[1]*Department of Bioscience, University of Birmingham, Edgbaston, B15 2TT, UK; E-mail: anna_vecchione@hotmail.com*
[2]*Department of Pediatrics, Medical University of South Carolina, 221 Fort Johnson Road, Charleston, SC 29412, USA*
[3]*Marine Biomedicine and Environmental Science Center, Medical University of South Carolina, 221 Fort Johnson Road, Charleston, SC 29412, USA*
[4]*Department of Research and Veterinary Services, Mystic Aquarium and Institute for Exploration, 55 Coogan Boulevard, Mystic, CT 06355, USA*
[5]*National Oceanic and Atmospheric Administration/National Ocean Service/Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC 29412-9110, USA*

Abstract

Bottlenose dolphins (*Tursiops truncatus*) are top-level marine predators and represent a sentinel species for ocean health. The study of cytokines and immunocellular function are important components for assessing the overall health status of wild dolphins. Recent studies have generated an increased number of identified cytokine molecules in *T. truncatus*, which will help in furthering health assessment in this species as they can be used in vitro to determine how pollutants or environmental stressors can influence plasma cytokine expression profiles, cell proliferation, and cytokine gene expression in tissues. Additionally, identification of *T. truncatus* immune modulators is important as the use of nonspecies-specific cytokines in some assays could lead to ambiguous results. Herein, the authors review some of the recent findings regarding *T. truncatus* cytokines such as interleukin (IL)-1, IL-2, IL-4, IL-8, interferon gamma (IFN-γ), and tumor necrosis factor (TNF-α), and indicate the possible use of Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) for in vitro qualitative and quantitative production of macrophage granulocyte lineages in bottlenose dolphins. Finally, the authors underline the importance of cytokine biological activity to obtain an accurate evaluation of the bottlenose dolphin immuno-physiological status in relation to environmental contamination.

Key Words: cytokines, bottlenose dolphin, *Tursiops truncatus*, marine mammal, immune function

Introduction

Cytokines are a group of soluble proteins or glycoproteins involved in the communication between different kinds of cells. They can be secreted, held in reservoir in the extracellular matrix, or expressed on the cell surface membrane. Most have more than one action. They are formed through the activation of innate and acquired immunity, initiate the inflammatory response, and are responsible for the extent and nature of the acquired immune response to certain stimuli. Cytokines, such as interleukin (IL)-1, IL-2, IL-4, IL-7, and IL-9 are responsible for proliferation and differentiation of T-cells, B-cells, and macrophages, which are involved in acquired immunity. IL-3, Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), and IL-5 are involved in the proliferation and differentiation of neutrophils, eosinophils, macrophages, and mast cells, which are implicated in innate immunity (Nicola, 1994; Fitzgerald et al., 2001).

In pinnipeds and cetaceans, the relevance of biological properties and molecular analysis of cytokines has been only recently addressed. For example, the importance of IL-2, IL-10, and interferon gamma (IFN-γ) as regulators for immune function in marine mammals has been reviewed (Bradley & Reynolds, 2002; King & Stott, 2002). Similarly, IL-1, IL-6, and colony stimulating factors have been considered as possible indicators of inflammatory diseases and for therapeutic use in marine mammals (King & Stott, 2002). The effect of exposure to pollutants on transcription of cytokines and their plasma levels has been evaluated in different species. In the North and Baltic Seas, for harbor porpoises (*Phocoena phocoena*) exposed to polychlorinated biphenyls (PCBs) and

polybrominated diphenyl ethers (PBDEs), lymphoid depletion in the thymus and spleen was associated with impaired health status (Beineke et al., 2007). In lymphoid depleted subjects, evaluation of gene expression in the blood indicated only up-regulation of the anti-inflammatory cytokine IL-10, while RT-PCR analysis for IL-2, IL-4, IL-6, and tumor necrosis factor-α (TNF-α) showed normal gene expression (Beineke et al., 2007). Alternatively, in peripheral leukocytes of harbor seals (*Phoca vitulina*), down-regulation of transcription for IL-2 and IL-1 has been detected following the exposure to polycyclic aromatic hydrocarbons (PAHs) and PCBs (Neale et al., 2005). In humans, significant dose-dependent changes in plasma cytokine levels were not found following the exposure of individuals to PCBs and hexachlorobenzene (HCB) with the exception of IFN-γ plasma levels that were decreased in subjects exposed for long periods of time to HCB (Daniel et al., 2001a). Nonetheless, significant dose-dependent humoral and cellular immunodeficiencies can be found in humans exposed to other pollutants (Daniel et al., 2001b) as in the case of the pesticide pentachlorophenol (PCP).

Little is known about cytokine signaling control of cell proliferation, differentiation, migration, survival, and regulation of the immune response in the bottlenose dolphin. Knowledge of the role of colony stimulating factors and cytokines in bottlenose dolphins' innate and acquired immunity in the presence of external contaminants or disease states is limited. A cytokine panel has been suggested as part of an immunological screening-set for the assessment of bottlenose dolphin health (Peden-Adams & Romano, 2005), but to date this is unavailable.

Current State of Knowledge on Cytokines in Tursiops truncatus

Information on *T. truncatus* IL-8, IL-4, IL-2, IL-1 (α and β), TNF-α, and IFN- γ is accessible in the literature (McMahon, 1996; Inoue et al., 1999a, 1999b, 1999c; Shoji et al., 2001; Bradley & Reynolds, 2002; King & Stott, 2002). A *T. truncatus* micro-array that contains IL-1R, IL-8R, GM-CSF, IL-6, IL-12, IL-16, IL-17, IL-10, IL-13, and TGF-β is also available (Mancia et al., 2007). The partial sequences for the cytokines and cytokine receptors on the array, although not currently published, are available in GenBank as ESTs and at the Marine Genomics Project website (www.marinegenomics.org).

Only some of the cytokines belonging to *T. truncatus* have been cloned or their complete amino acid sequence obtained, and of these, few have had their biological activity tested in tissue culture. In a few cases, mitogens have been used to obtain information on cytokines as shown in a study by Erickson et al. (1995) in *T. truncatus* on IL-2 receptor and peripheral blood mononuclear leukocyte proliferation assays. In a number of studies, including marine mammal studies, scientists have relied on cross-reactivity of commercially available cytokines and reagents among the species. For example, cross-reactivity of recombinant human IL-2 in marine mammal cell culture systems has been detected. Recombinant human IL-2 increases natural killer cell activity in harbor seals (Ross et al., 1996) and beluga whales (*Delphinapterus leuca*) (De Guise et al., 1997). However, it is not fully established if biological activity of human IL-2 in *T. truncatus* cells is equivalent or similar to that detected in human cells.

Potential Issues with Use of Nonspecies-Specific Cytokines

Findings in other species indicate that in some cases heterologous cytokines cannot be used in studies for quantitative and qualitative analysis of cellular signaling and functions. Use of cytokines from one species on cells of another can lead to an incomplete signal, resulting in either incomplete or no biological activity (Leong et al., 1989; Mire-Sluis & Thorpe, 1998). For the species in which cytokines have been described, they may have high similarity in their nucleotide and amino acid sequence. However, regardless of having common sequences or similar characteristics of receptor signaling, each cytokine has a specific range of biological functions *in vivo*. Even small differences in identity can have a strong effect on the binding of the cytokine to cytokine receptors. The biological outcome of cytokine stimulation is related to a number of factors such as the exact combination of ligand and receptor. Alteration of the biological activity of the cytokine can also be caused by a single amino acid change, even though the amino acid is not directly involved in receptor binding (Altmann & Kastelein, 1995).

For example, in the case of cytokines like GM-CSF, the binding of the fourth alpha-helix of mouse GM-CSF to the alpha subunit of the receptor complex is compromised by a few amino acid mutations (Altmann & Kastelein, 1995). In the case of other cytokines, such as human oncostatin M (OSM), the ligand binds to its receptor and also to the human Leukemia Inhibitor Factor-receptor (LIF-R). In contrast, murine OSM binds only to the OSM-receptor, yet murine LIF can bind to the human LIF-R with low affinity (Layton et al., 1994; Robinson et al., 1994; Owczarek et al., 1997). The substitution of just a few amino acids, such as the residues phenylalanine or tryptophan and adjacent arginine residues, can determine changes in the capacity of receptor recognition of IL-6, IL-11, LIF, and OSM. Furthermore,

the structures of ligands are directly related to their receptors as demonstrated by a hydrophobic area of the insulin receptor which binds to an exposed non-polar site of insulin (Layton et al., 1994; Bravo & Heath, 2000). Moreover, use of the A375 IL-1 sensitive cell line is not specific enough to assess Florida manatee IL-1 production from mitogen simulated PBLs (C. Walsh, pers. comm.), suggesting that Florida manatee IL-1 does not cross-react well with the IL-1R of this standard cell line. Consequently, these findings suggest that some non-heterologous cytokines may not be a reliable tool for the analysis of immunocellular function in *T. truncatus*.

Percentage Identity Between Some T. truncatus *Cytokines and Homologs of Different Species*

A comparison of *T. truncatus* cytokines with those of marine mammal species was performed to determine which species has the highest percentage identity to the dolphin. Establishment of which species may have preeminent cytokine cross-reactivity for *T. truncatus* could support immunological studies when a specific cytokine is needed but is not available. The amino acid sequences for *T. truncatus* IL-1α, IL-1β (Inoue et al., 1999c), IL-2 (Bradley & Reynolds, 2002), IL-4 (Inoue et al., 1999a), IL-8 (Itou et al., 2003), IFN-γ (Inoue et al., 1999b), and TNF-α (Shoji et al., 2001) were aligned with cytokines from other species and evaluated using the NCBI *Basic Local Alignment Search Tool* (*BLAST*) protein database search programs to help to establish functional and evolutionary relationships between sequences (Altschul et al., 1990, 1997; Madden et al., 1996; Zhang & Madden, 1997). Using the *BLAST* method analysis, no consistent higher percentage identity of the *T. truncatus* cytokines IL-1, IL-2, IL-4, IL-8, IFN-γ, or TNF-α was found with homologos cytokines from other species. However, the percentage identity of cytokine sequences from some species that appeared to be closer to *T. truncatus* in the *BLAST* analysis is shown in Table 1.

Tumor Necrosis Factor-Alpha—Macrophages express TNF-α in combination with other cytokines such as G-CSF, GM-CSF, and IL-1 (Metcalf, 1984). TNF-α induces fibroblasts to produce GM-CSF, which could have a role in the maturation of granulocyte and macrophage progenitors (Metcalf, 1980; Mellstedt et al., 1999). Evaluation of TNF-α function and production in bottlenose dolphins may be highly important considering its vital role in other species. In humans, TNF-α inhibition causes decreased host-resistance to microorganisms such as mycobacteria and fungi (Hamilton, 2005). Similar to human neutrophils, bottlenose dolphin TNF-α has a pivotal role in increasing respiratory burst oxidase and consequently microbicidal activity of dolphin neutrophils (Itou et al., 2002). Furthermore, this cytokine has an important function in the inflammation processes as a non-inflammatory steady-state may depend on the balance of TNF-α expression levels. For example, in the horse (*Equus caballus*), TNF-α free radicals, prostaglandin E2, and IL-1 are some of the inflammatory mediators, which can be found with damaged hyaluronan in synovial fluid and articular cartilage (Frisbie & McIlwraith, 2001). It would be interesting to establish if this finding can also be applied to dolphins presenting spinal cord motor disturbances. Evaluation of transcript of TNF-α is also useful to determine if the translation repression system in which IL-4, IL-10, and TGF-β are involved is functional (Ebert, 2005).

TNF-α has not only been identified and sequenced in *T. truncatus* (Shoji et al., 2001),

Table 1. Percentage of amino acid sequences' identity for *T. truncatus* cytokines versus homologs from other species; *T. truncatus* IL-8 from Itou et al. (2003) and IL-1α, IL-1β, and Interferon-γ from Inoue et al. (1999b, 1999c) versus homologs of several terrestrial species are shown. The *T. truncatus* IL-2 nucleotide sequence obtained from Bradley & Reynolds (2002) was submitted in NCBI *BLAST* as translated query versus translated database and amino acid sequence percentage identity acquired. Sequence alignments were obtained with protein database search programs of NCBI (Altschul et al., 1990, 1997; Madden et al., 1996; Zhang & Madden, 1997). For amino acid sequences' accession numbers, see Table 3.

Cytokines	*T. truncatus* GenBank accession numbers	Percent amino acid sequence similarities			
		Ovine	Bovine	Swine	Human
Interleukin-1α	BAA87946	77%	78%	76%	65%
Interleukin-1β	BAA87947	76-77%	76%	69-73%	64%
Interleukin-2	Not available	75%	76%	77%	77%
Interleukin-4	Q9XS58	76%	77%	87%	59%
Interleukin-8	BAC81421	89%	88%	86%	75%
Interferon-γ	BAA82042	86%	86%	80%	63%
Tumor necrosis factor-α	BAB39855	82%	84%	85%	83%

but also in other marine mammals such as beluga whales (Denis & Archambault, 2001), rough-toothed dolphins (*Steno bredanensis*) (ABC68490), and the Florida manatee (*Trichechus manatus latirostris*) (ABC58216) (Table 2). High similarity in TNF-α amino acid sequences among *T. truncatus*, *D. leucas*, and *S. bredanensis* is apparent, while lower amino acid similarity can be detected in *T. m. latirostris*. Bottlenose dolphin recombinant TNF-α cross-reacts with an anti-human TNF-α antibody and is biologically active on the murine cell line L929 (Shoji et al., 2001). Nonetheless, the amino-acid sequence of bottlenose dolphin TNF-α compared to mouse homologos (P06804) has only 74% identity.

Interferon-Gamma—In numerous species, antiviral and immuno-modulatory activities of the interferons (IFNs) for innate immunity as first line of host defense have been described (La Bonnardiere et al., 1994; Decker et al., 2002; Schroder et al., 2004; Takaoka & Yanai, 2006). In innate immunity, IFN subfamilies have clear roles against viruses, bacteria, and protozoa as well as having a role in networking between innate and adaptive defense (Mannering & Deloria, 1986; Decker et al., 2002). In particular, IFN-γ can modulate specific immune responses as it is involved in monocyte differentiation from dendritic cells to macrophages (Delneste et al., 2003); can enhance expression of TNF receptors (Tsujimoto et al., 1986); and can up-regulate the production of GM-CSF, IL-1α, and TNF-α in keratinocytes (Pastore et al., 1998). In *T. truncatus*, the three IFN type I subfamilies (alpha, beta, and omega) are not available in NCBI accession Genbank. However, the *T. truncatus* IFN-γ (type II IFN subfamily) is available (BAA82042) (Inoue et al., 1999b). The comparison of percentage identity for *T. truncatus* IFN-γ amino acid sequences with that of other species showed that human is the least similar in amino acid identity with 63% while higher identity can be detected between bottlenose dolphin and ovine (86%) (Table 1). Due to the importance of this cytokine in innate immunity, further studies on IFNs of the family Delphinidae are essential to better comprehend how innate-adaptive immunity networking could be affected by anthropogenic stressors and pollutants.

Interleukin 1—IL-1α and β play not only an important role in the initiation of inflammatory events but are also involved in the control of infectious diseases. In particular, IL-1α participates in cell growth and contributes to repair processes (Dinarello, 1994). Since expression levels of IL-1 can increase following the release of stress hormones (Calcagni & Elenkov, 2006) and as IL-1 has an important function in both acute and chronic inflammation (Dinarello, 1994; Feghali & Wright, 1997), evaluation of plasma expression levels of this cytokine may be an additional tool in the assessment of the general health of a free-ranging dolphin. Cloning and sequencing of IL-1α (BAA87946) and IL-1β (BAA87947) in *T. truncatus* has been reported in the NCBI database (Inoue et al., 1999c). Specific in vitro immuno-assays have shown that IL-1β cytostatic activity can be inhibited in human melanoma cells pre-treated with dolphin recombinant IL-1 receptor antagonist (Inoue et al., 2001). Nonetheless, the bottlenose dolphin IL-1β sequence, when compared with humans exhibited only 64% identity and with other terrestrial species, such as bovine and ovine, showed to be 76 to 77%, respectively (Table 1).

Tursiops truncatus IL-1β (BAA87947) when aligned with *D. leucas* IL-1β (Denis & Archambault, 2001) presents 96% identity in amino acid sequence. Amino acid identity of *Tursiops* IL-1β with both Pacific harbor seal (*Phoca vitulina richardsi*) (AAS91558) and ringed seal (*P. hispida*) (ABC87314) was 65%, and with Florida manatee (*T. m. latirostris*) (AAW63042) was 64%. Evaluation of IL-1α percentage identity among *T. truncatus* and other marine mammals was not possible due to the absence of complete amino acid sequences for these cytokines in the NCBI database.

Interleukin 4, Interleukin 2, and Interleukin 8—Mediation of humoral and cellular responses by IL-4 and influence of IL-2 on cellular responses have considerable roles in chronic inflammation (Feghali & Wright, 1997). IL-4 in association with GM-CSF can preserve the function and viability of dendritic cells, which are important for their role in induction of the primary immune response (Sallusto & Lanzavecchia, 1994). The complete sequence of *T. truncatus* IL-4 has been attained (Inoue et al.,

Table 2. Percentage amino acid identity between *T. truncatus* TNF-α and homologs of some marine mammals

Marine mammal species	TNF-α amino acid percentage identity with bottlenose dolphins	
	GenBank accession numbers	TNF-α
Delphinapterus leucas	AAL56946	96-98%
Steno bredanensis	ABC68490	99%
Trichechus manatus latirostris	ABC58216	83%

1999). It presents only 59% identity in amino acid sequence when compared with humans; however, percentage identity is represented by 87% when compared to swine (Table 1). Sequences of IL-4 in other marine mammals have also been reported for harbor seals (AAY52233) and harbor porpoises (AAK19740); unfortunately, as only partial amino acid sequences are available, a complete evaluation cannot be performed in the case of this particular cytokine. IL-2 family cytokines also influence survival-proliferation signaling pathways in T-lymphocytes (Benczik & Gaffen, 2004). In harbor seal pups, changes of lymphocyte proliferation are associated with the presence of organic pollutants in the environment (Levin et al., 2005). In the bottlenose dolphin, however, the correlation between IL-2 expression, organic pollutants, and lymphocyte viability needs further investigation.

IL-8 mainly plays an important role in acute inflammation and is a T-lymphocyte and neutrophil chemotactic activating factor. This cytokine is rapidly released following microbial and non-microbial agent invasion. In humans, plasma concentration of IL-8 is commonly evaluated in the diagnosis of neonatal bacterial infection (Volante et al., 2004). In bovine macrophages, IL-8 mRNA synthesis is up-regulated as early as 1 h in the presence of *Pasteurella haemolytica* or *Mannheimia haemolytica* (Morsey et al., 1996), but increased expression levels of IL-8 lasts no more than 24 h in bovine innate immune response following *Pseudomonas aeruginosa mastitis* (Bannermann et al., 2005). Therefore, it is possible that quantification of the expression level of this cytokine may not be a consistent method for the investigation of microbial infections in marine mammals. IL-8 is also an angiogenic factor and, in human melanoma, its over-expression is a clear sign of metastasis (Melnikova & Bar-Eli, 2006). However, in bottlenose dolphins, it has not yet been established that the presence of a neoplastic growth corresponds with over-expression of IL-8. The amino acid sequence identity for *T. truncatus* IL-8 showed identity to homologos sequences from ovine, bovine, and swine of 86 to 89%, while only 75% was detected when compared to that of humans (Table 1), corroborating the hypothesis that artiodactyls in general appear to be the most closely related to cetaceans (Gingerich et al., 1983; Bradley & Reynolds, 2002).

In *T. truncatus*, isolation and expression of cDNA encoding IL-2 has been shown (McMahon, 1996; King & Stott, 2002) and cytokine sequence similarity appraised among species (Bradley & Reynolds, 2002). IL-2 nucleotide sequence by Bradley & Reynolds showed 98% amino acid identity between bottlenose dolphins and IL-2 of killer whales (*Orcinus orca*) (Ness et al., 1998). Approximately 96% identity was detected between amino acid sequences of IL-2 of *T. truncatus* and *D. leucas* (St-Laurent et al., 1999). There is a high level of homology in nucleotide sequence for IL-2 among the members of the Delphinidae family (Bradley & Reynolds, 2002). Comparison of *T. truncatus* IL-2 nucleotide sequence with the homologs of other non-marine mammal species demonstrates that swine are more closely related to dolphins than other terrestrial mammals. The findings of Bradley & Reynolds for *T. truncatus* IL-2 have confirmed Gatsey's (1997) implications that suborder Suiformes are the most related to cetaceans. On the other hand, in this study, the percentage identity of amino acid sequence for IL-1, IL-2, IL-4, IL-8, IFN-γ, or TNF- α indicates that no particular terrestrial species has a significant and consistent high percentage identity with the cytokines of bottlenose dolphins (Table 1).

Current and Future Research Needs

Although previous studies in some marine mammals have highlighted the immuno-suppressant effect of pollutants (Ross et al., 1996; Levin et al., 2005, 2007; Mos et al., 2006; Bossart, 2006), further investigations and tools are needed to better understand how the host immune system can be so radically affected by them. Detection of cytokines with hematopoietic activities and their cloning may allow the application of in vitro screening

Table 3. Accession numbers of amino acid sequences for ovine, bovine, swine, and human cytokines used to identify percentage identity of sequences with homologs bottlenose dolphin cytokines

Cytokines	Bottlenose dolphin	Ovine	Bovine	Swine	Human
Interleukin-1α	BAA87946	NP001009808	NP776517	NP999194	CAA27448
Interleukin-1β	BAA87947	CAA38566	NP776518	NP999220	AAA59135
Interleukin-2	Not available	NP001009806	AAA30586	NP999026	NP000577
Interleukin-4	Q9XS58	P30368	AAA82730	CAA48407	AAA59150
Interleukin-8	BAC81421	NP001009401	NP776350	BAC06611	AAA59158
Interferon-γ	BAA82042	NP001009803	NP776511	CAA37252	AAB59534
Tumor necrosis factor-α	BAB39855	CAA39437	NP776391	NP999187	NP000585

with immune assays where a specific cell lineage is required. For example, studies on dendritic cells, macrophages, and granulocytes with their toll-like receptors (TLRs) would be possible with the cloning of GM-CSF from *T. truncatus*. The macrophage and granulocyte lineages are the first lines of defense against infectious pathogens. Their TLR signals induce production of pro- and anti-inflammatory cytokines with the creation of networking between innate responses and specific immune responses (Ozato et al., 2002). Investigations of cytokine networking will help in the understanding of *T. truncatus's* innate and adaptive immune responses towards pathogens and how the immune defense can be influenced by pollutants. In terrestrial species, IL-1 and TNF-α released by activated macrophages stimulates endothelial cells to produce GM-CSF, G-CSF, and M-CSF (Krishnaswamy et al., 1999; Barreda et al., 2004). Up-regulation of GM-CSF production following stimulation by cytokines or antigens such as bacterial cell walls results in the induction of hematopoietic events in bone marrow. These result in increased mobilization of effector cells of the macrophage and granulocyte lineages to the peripheral blood and the induction of functional responses in mature cells at the inflammatory site or throughout the body (Barreda et al., 2004). GM-CSF in conjunction with IL-4 expands myeloid dendritic cells and preserves their function (Sallusto & Lanzavecchia, 1994). Therefore, identification and sequencing of GM-CSF in *T. truncatus* could be a useful tool for in vitro qualitative and quantitative production of macrophage and granulocyte lineages and to establish how these cells function can be affected by pollutants.

Several other cytokines, such as IL-6, IL-10, and IL-12, should be identified and sequenced for the development and production of reagents for in vitro *T. truncatus* studies. The information obtained by antibody detection of the expression level of these cytokines is important during autoimmunity, chronic infections, and susceptibility to diseases caused by stress. Stress hormones influence expression levels of IL-6, IL-10, and IL-12, causing an imbalance between pro- and anti-inflammatory Th1/Th2 cytokines and, consequently, increasing disease susceptibility in the stressed subject (Calcagni & Elenkov, 2006). Cytokine expression levels in wild dolphins could be determined with available dolphin Th1/Th2 cytokine sequences or partial sequences by utilizing RT-PCR. Evaluation of expression levels of Th1/Th2 cytokines in wild dolphins in contact with environmental stressors could be an additional tool to further demonstrate the association between a compromised environment and disease in this species.

The evaluation of hematology, biochemical, and cytology parameters in *T. truncatus* together with studies on emerging diseases has recently expanded the assessment of health in wild populations (Bossart et al., 2006; Fair et al., 2006; Goldstein et al., 2006; Reif et al., 2006). Information on their hematopoietic system and cytokine level is crucial to a better understanding of health status and immunological function. Since hematopoietic growth factors can have major impacts on the immune response (Mellstedt et al., 1999), it is important to establish how these may differ in *T. truncatus* from the homologs of other species and to determine how growth factors regulate progenitor cell differentiation. Classification of lineage-specific surface molecules in the bottlenose dolphin to identify pluripotent and differentiated blood cells and their cellular activity may be a link that helps reveal that immunity can be influenced by pollutants and other environmental stressors (Romano et al., 2006).

Conclusion

To obtain a more comprehensive evaluation of the health status and condition of immunological function in *Tursiops truncatus*, it is important to obtain additional information on this species' hematopoietic system and cytokine function. In some cases, the use of heterologous cytokines in vitro can produce unreliable assay results as biological activity of a nonspecies-specific cytokine could be incomplete in *T. truncatus* cells. The percentage identity among some *T. truncatus* cytokines and those of different species suggests that several members of the order artiodactyla are closely related to this marine mammal. Since the human shows the least percentage identity with *T. truncatus* cytokines, the use of human cytokines is not highly recommended in immuno-assays that are used for *T. truncatus*. Consequently, the use of nonspecies-specific cytokines for quantitative and qualitative analysis of cellular functions and signaling is not suggested unless their complete biological activity on *T. truncatus* cells can be demonstrated. The immune system of *T. truncatus* remains the subject of significant research interest, and several different techniques are being utilized to define its progressive development (Erickson et al., 1995; DeGuise et al., 1997; Fair & Bossart, 2006; Fair et al., 2006; Goldstein et al., 2006). This species may be an important indicator/sentinel of ocean health (Bossart, 2006). Therefore, investigation into the roles of cytokines in innate and adaptive immune-defense mechanisms that are involved in disease processes and their relations to environmental stressors is necessary.

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