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Immunocytes of the Atlantic bottlenose dolphin (*Tursiops truncatus*) and West Indian manatee (*Trichechus manatus latirostris*) : morphologic characterizations and correlations between healthy and disease states under free-ranging and captive conditions

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FLORIDA INTERNATIONAL UNIVERSITY

MIAMI, FLORIDA

IMMUNOCYTES OF THE ATLANTIC BOTTLENOSE DOLPHIN

(TURSIOPS TRUNCATUS)

AND WEST INDIAN MANATEE

(TRICHECHUS MANATUS LATIROSTRIS):

MORPHOLOGIC CHARACTERIZATIONS AND CORRELATIONS
BETWEEN HEALTHY AND DISEASE STATES UNDER FREE-RANGING
AND CAPTIVE CONDITIONS

A dissertation submitted in partial satisfaction

for the degree of

DOCTOR OF PHILOSOPHY IN BIOLOGICAL SCIENCES

by

Gregory D. Bossart, V.M.D.

1995

To: Arthur Herriott
College of Arts and Sciences

This dissertation, written by Gregory D. Bossart, and entitled "Immunocytes of the Atlantic Bottlenose Dolphin (Tursiops truncatus) and West Indian Manatee (Trichechus manatus latirostris): Morphologic Characterizations and Correlations Between Healthy and Disease States under Free-ranging and Captive Conditions", having been approved in respect to style and intellectual content, is referred to you for judgement.

We have read this dissertation and recommend that it be approved.

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Florida International University, 1995

This work is dedicated to:

- ~ my mother, Lois Gregory Capuder, who gave me love and encouragement throughout the pursuit of my dreams,
- ~ Jennifer L. Kimbrell, who reintroduced me to the joy and love of God,
- ~ and, of course, to manatees and dolphins, for some of the best years of my life.

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ABSTRACT OF THE DISSERTATION

IMMUNOCYTES OF THE ATLANTIC BOTTLENOSE DOLPHIN
(TURSIOPS TRUNCATUS) AND WEST INDIAN MANATEE (TRICHECHUS
MANATUS LATIROSTRIS): MORPHOLOGIC CHARACTERIZATIONS
AND CORRELATIONS BETWEEN HEALTHY AND DISEASE STATES
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Gregory D. Bossart, V.M.D.

Florida International University, 1995

Professor Charles Bigger, Major Professor

Interest in the health of marine mammals has increased due, in part, to the attention given to human impact on the marine environment. Recent mass strandings of the Atlantic bottlenose dolphin (Tursiops truncatus) and rising mortalities of the endangered Florida manatee (Trichechus manatus latirostris) have raised questions on the extent to which pollution, infectious disease, "stress," and captivity influence the immune system of these animals. This study has provided the first in-depth characterization of immunocytes in the peripheral blood of dolphins (n=180) and manatees (n=56). Immunocyte morphology and baseline values were determined in clinically normal animals under free-ranging, stranded and captive living conditions as well as by age and sex. Additionally, immunocyte population dynamics were characterized in sick animals. This was

accomplished with traditional cytochemical techniques and new lymphocyte phenotyping methodology which was validated in this study. Traditional cytochemical techniques demonstrated that blood immunocyte morphology and cell numbers are similar to terrestrial mammals with some notable exceptions. The manatee heterophilic granulocyte is a morphologically unique cell and probably functions similarly to the typical mammalian neutrophil. Eosinophils were rarely found in manatees but were uncommonly high in healthy and sick dolphins. Basophils were not identified. Manatees had higher total lymphocyte numbers compared to dolphins and most terrestrial mammals. Lymphocyte subsets identified in healthy animals included T_h , T_{cs} , B and NK cells. Dolphin and manatee T and B cell values were higher than those reported in man and most terrestrial mammals. The manatee has extraordinarily high absolute numbers of circulating T_h cells which suggests an enhanced immunological response capability. With few exceptions, immunocyte types and absolute numbers were not significantly different between free-ranging, stranded and captive categories or between sex and age categories. The evaluation of immunocyte dynamics in various disease states demonstrated a wide variation in cellular responses which provided new insights into innate, humoral and cell-mediated immunity in these species. Additionally, this study demonstrated that lymphocyte phenotyping has diagnostic significance and could be developed into a potential indicator of immunocompetence in both free-ranging and captive dolphin and manatee populations.

TABLE OF CONTENTS

CHAPTER		PAGE
1.00	INTRODUCTION	1
2.00	MATERIALS AND METHODS	II
2.10	STUDY POPULATION	11
2.20	ENVIRONMENTAL CATEGORIES	12
2.30	HEALTH CATEGORIES	13
2.40	AGE AND SEX CATEGORIES	14
2.50	SAMPLE COLLECTION	16
2.51	PERIPHERAL BLOOD	16
2.52	LYMPHOID TISSUE	17
2.53	MURINE LYMPHOID TISSUE - POLYMETHINE STAIN VALIDATION STUDY	17
2.60	SAMPLE ANALYSES	20
2.61	COMPLETE BLOOD COUNT WITH TRADITIONAL IMMUNOCYTE DIFFERENTIAL CELL COUNTS FROM PERIPHERAL BLOOD	20
2.62	POLYMETHINE-STAINED LYMPHOCYTE SUBSET DIFFERENTIAL CELL COUNTS FROM PERIPHERAL BLOOD	21
2.63	POLYMETHINE-STAINED LYMPHOID TISSUE PREPARATIONS	23
2.64	HISTOPATHOLOGIC EVALUATION OF LYMPHOID TISSUE	24
2.70	STATISTICAL EVALUATIONS	24

CHAPTER	PAGE
3.00 RESULTS	25
3.10 DOLPHIN IMMUNOCYTE MORPHOLOGY - WRIGHT-GIEMSA STAINED PERIPHERAL BLOOD	25
3.20 MANATEE IMMUNOCYTE MORPHOLOGY - WRIGHT-GIEMSA STAINED PERIPHERAL BLOOD	28
3.30 MURINE LYMPHOID TISSUE - POLYMETHINE STAIN VALIDATION STUDY	32
3.40 DARKFIELD MICROSCOPIC EXAMINATION OF POLYMETHINE-STAINED PERIPHERAL BLOOD PREPARATIONS FROM DOLPHINS AND MANATEES	33
3.50 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS	41
3.51 HEALTH AND ENVIRONMENTAL CATEGORY IMMUNOCYTE DYNAMICS - DOLPHINS	41
3.52 AGE AND SEX IMMUNOCYTE DYNAMICS - DOLPHINS	45
3.53 HEALTH AND ENVIRONMENTAL CATEGORY IMMUNOCYTE DYNAMICS - MANATEES	47
3.54 AGE AND SEX IMMUNOCYTE DYNAMICS -MANATEES	50
3.55 IMMUNOCYTE DYNAMICS - INDIVIDUAL SERIAL CASE STUDIES.....	51
3.60 LYMPHOID TISSUE EVALUATION	51
3.61 POLYMETHINE-STAINED TOUCH PREPARATIONS ..	51
3.62 HISTOPATHOLOGIC EVALUATION OF LYMPHOID TISSUES	52

CHAPTER	PAGE
4.00 DISCUSSION	59
4.10 IMMUNOCYTE CHARACTERIZATION	59
4.11 MORPHOLOGY AND VALUES OF IMMUNOCYTES IN HEALTHY DOLPHINS	59
4.12 MORPHOLOGY AND VALUES OF IMMUNOCYTES IN HEALTHY MANATEES	65
4.20 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS - A REVIEW	68
4.21 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS IN HEALTHY AND DISEASE STATES - DOLPHINS	78
4.22 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS IN HEALTHY AND DISEASE STATES - MANATEES	86
4.30 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS- INDIVIDUAL SERIAL CASE STUDIES.....	90
4.31 SERIAL CASE STUDIES - DOLPHINS.....	90
4.32 SERIAL CASE STUDIES - MANATEES	101
4.40 LYMPHOID TISSUE COMPARTMENT EVALUATION	108
5.00 CONCLUSIONS	112
5.10 GENERAL SUMMARY	112
5.20 RESEARCH NEEDS AND RECOMMENDATIONS	120
6.00 LITERATURE CITED	122

CHAPTER	PAGE
7.00 APPENDICES.....	146
APPENDIX A - HEMATOLOGIC AND HISTOPATHOLOGIC TABLES.....	147
APPENDIX B - STATISTICAL TABLES	170

LIST OF TABLES

TABLE		PAGE
2.710	Significantly different immunocyte parameters by health category in dolphins	171
2.720	Significantly different immunocyte parameters in captive-born (Cb) dolphins	172
2.730	Significantly different immunocyte parameters in captive-captured (Cc) dolphins	172
2.740	Significantly different immunocyte parameters in free-ranging stranded (Fs) dolphins	173
2.750	Significantly different immunocyte parameters in free-ranging (Ff) dolphins	173
2.760	Significantly different immunocyte parameters in healthy (H1) dolphins by living environment category	174
2.770	Significantly different immunocyte parameters in acutely-ill (H21) dolphins by living environment category	175
2.780	Significantly different immunocyte parameters in chronically-ill (H3) dolphins by living environment category	175
2.790	Significantly different immunocyte parameters in dolphins under altered physiologic conditions (H4) by living environment category	175
2.791	Significantly different immunocyte parameters in healthy (H1) dolphins by sex	176
2.792	Significantly different immunocyte parameters in acutely-ill (H21) dolphins by sex	176
2.793	Significantly different immunocyte parameters in dolphins under altered physiologic conditions (H4) by sex	176

TABLE	PAGE
2.794	Significantly different immunocyte parameters by health category in dolphins from 2 to 10 years-of-age (A2) 177
2.795	Significantly different immunocyte parameters by health category in dolphins over 10 years-of-age (A3) 178
2.796	Significantly different immunocyte parameters by health category in manatees 179
2.797	Significantly different immunocyte parameters in free-ranging injured (Fs) manatees 180
2.798	Significantly different immunocyte parameters by health category in manatees from 2 to 10 years-of-age (A2) 181
3.511	Comparison of living environment status in clinically normal dolphins 148
3.512	Comparison of living environment status in acutely ill dolphins with probable bacterial infections 149
3.513	Comparison of living environment status in acutely ill dolphins with terminal septicemia and/or viral infection 150
3.514	Comparison of living environment status in chronically ill dolphins 151
3.515	Comparison of living environment status in dolphins under altered physiologic conditions 152
3.521	Comparison of sex status vs. health status in dolphins 153-154
3.522	Comparison of age vs. health status in dolphins 155-156
3.531	Comparison of living environment status in clinically normal manatees 157

TABLE		PAGE
3.532	Comparison of living environment status in acutely ill manatees with probable bacterial infections	158
3.533	Comparison of living environment status in acutely ill manatees with terminal septicemia and/or viral infection	158
3.534	Comparison of living environment status in manatees under altered physiologic conditions	159
3.541	Comparison of sex status vs. health status in manatees	160
3.542	Comparison of age vs. health status in manatees	161-162
3.551	Immunocyte dynamics - Serial case studies - Dolphins	163-164
3.552	Immunocyte dynamics - Serial case studies- Manatees	165
3.621	Summary of lymphoid tissue histopathologic evaluations	166
3.622	Graded histopathologic lymphoid tissue results in dolphins .	167-168
3.623	Graded histopathologic lymphoid tissue results in manatees	169

LIST OF PLATES

PLATE		PAGE
3.101	Dolphin immunocytes. Wright-Giemsa stain.	27
3.201	Manatee immunocytes. Wright-Giemsa stain.	30
3.202	Manatee heterophilic granulocytes and eosinophil. Wright-Giemsa stain.	31
3.401	Dolphin immunocytes. Polymethine stain.	36
3.402	Dolphin B lymphocyte. Polymethine stain.	37
3.403	Dolphin T lymphocyte subsets. Polymethine stain.	38
3.404	Manatee NK cell. Polymethine stain.	39
3.405	Manatee T lymphocyte subsets. Polymethine stain.	40
3.621	Histologic architecture of a normal dolphin lymph node.	54
3.622	Histologic architecture of dolphin lymphoid depletion.	55
3.623	Histologic architecture of dolphin morbillivirus lymphoid depletion.	56
3.624	Manatee lymph node lymphoid hyperplasia.	57
3.625	Higher power of manatee lymphoid hyperplasia.	58
5.101	Manatee human - related traumatic injury.	119

ABBREVIATIONS

A1	Less than or equal to ($\leq$) 2 years-of-age
A2	2 to 10 years-of-age
A3	Over 10 years of age
B	B lymphocyte
Cb	Captive-born living environment category
Cc	Captive-captured living environment category
EDTA	Dipotassium salt of ethylenediaminetetraacetic acid
Ff	Free-ranging living environment category
Fs	Free-ranging stranded or injured living environment category
H1	Clinically normal health category
H3	Chronically ill health category with normal or abnormal total leukocyte count (>8 weeks duration)
H4	Altered physiologic condition health category
H21	Acutely ill health category with elevated total leukocyte count (< 8 weeks duration)
H22	Acutely ill health category with decreased total leukocyte count (<8 weeks duration)
H&E	Hematoxylin and eosin
IL	Interleukin
mAb	Monoclonal antibody
NK	Natural Killer cell

ABBREVIATIONS

$T_{c/s}$	T cytotoxic-suppressor lymphocyte
T_h	T helper lymphocyte
T_h1	T_h1 subset cell
T_h2	T_h2 subset cell
μm	Micrometer

1.00 INTRODUCTION

Many aspects of the immune system of terrestrial mammals are well documented, particularly in humans and mice. Conversely, very little is known about the immune system in marine mammals, especially the exclusively aquatic cetaceans and sirenians, despite the fact that numerous species have been maintained in captivity for over forty years (National Marine Fisheries Service, 1990). The cetaceans include dolphins, whales and porpoises while the sirenians include three endangered manatee species and the dugong. The cetaceans are thought to have evolved from common ancestors of terrestrial ungulates and carnivores (Slijper, 1962) while the manatees are thought to share common ancestry with the elephant (Van Meter, 1987). Both cetaceans and sirenians are theorized to have left the land from 55-60 million years ago for an aquatic life (Gingerich, et al., 1983). This evolutionary history raises speculation of what adaptations, if any, have occurred in the cetacean and sirenian immune systems during the transition from a terrestrial to aquatic life.

The Atlantic bottlenose dolphin (Tursiops truncatus) is probably the most common odontocete maintained in captivity and has occasionally been studied in field research (Wells and Scott, in press). In 1987, an unprecedented 900 Atlantic bottlenose dolphins died during a 7 month period along the East coast of the United States (Geraci, 1989). Although controversial, the primary cause of this mass mortality was initially attributed to a naturally-occurring biotoxin by Geraci (1989). It is now been proven with retrospective immunohistologic methodology that this die-off was due to the newly described immunotropic dolphin morbillivirus (Lipscomb, 1994). Dolphins necropsied from this die-

off frequently had multiple focal and/or systemic opportunistic infections and primary lymphoid lesions suggesting immunologic compromise. Toxicologic examination of tissues from these dolphins revealed abnormally high levels of known immune system-suppressing anthropogenic toxins (Geraci, 1989). In 1990, 1991 and 1993, similar bottlenose dolphin mass mortalities occurred in the northern Gulf of Mexico and were investigated by the National Marine Fisheries Service (Hansen, 1992). New polymerase chain reaction methodology recently attributed the 1993 die-off to the dolphin morbillivirus (AFIP, 1994). Patterns of disease and limited toxicologic data suggested possible secondary immunologic compromise in many of these dolphins as well (Medway, et al., 1990). In reality, the secondary opportunistic infections are particularly important in dolphin morbilliviral disease because one of the primary sites of action of the virus is the lymphoid tissue, causing suppression of immune function (Dungworth, 1993).

In addition, case reports in the literature indicate that free-ranging odontocetes (including Atlantic bottlenose dolphins) which beach themselves and some ill captive dolphins often have clinicopathologic signs consistent with a state of immunologic compromise (Bossart, 1982; Bossart, 1984; Bossart, et al., 1985; Walsh, et al., 1988; Bossart, et al., 1991).

The knowledge of the bottlenose dolphin immune system is limited. Less than a dozen published reports exist concerning dolphin immunology with essentially no information concerning immunocyte subtypes and immune function. Published data involves the reporting of crude baseline clinical hematologic data for captive husbandry

(Medway and Geraci, 1964; Medway and Geraci, 1965; Medway and Geraci, 1966; Geraci and Medway, 1974; Geraci and Sweeney, 1986; MacNeill, 1975; Sweeney and Ridgway, 1975; Medway and Geraci, 1986; Asper, et al., 1990; Bossart and Dierauf, 1990). Macrophage-mediated immunologic defense mechanisms in dolphins is ongoing unpublished research (Fenwick, et al., 1986) funded by the U.S. Navy.

Mumford, et al. (1975) reported that dolphin peripheral blood lymphocytes were stimulated by pokeweed (a human B lymphocyte mitogen) more than phytohemagglutinin (a human T cell mitogen). Another study (Colgrove, 1978) reported that concanavalin A (a human T cell mitogen) produced a greater response than either pokeweed or phytohemagglutinin. A third study (Hohn, et al., 1983) reported that dolphin sera contained more agglutinins for human B cells than for human T lymphocytes.

In a recent study, a cross-reactive antibody to major histocompatibility complex (MHC) class II molecules and an immunoglobulin-specific antiserum were used for identifying these molecular species on lymphocytes of dolphins (Romano, et al., 1992). In a circuitous manner and without identifying specific T lymphocyte subtypes, this study concluded for the first time that healthy dolphin peripheral blood lymphocytes were 85-90% T cells and 10-15% B cells. Another recent study evaluated cellular immune responses in dolphins via an interleukin-2 receptor expression assay (Erickson, et al., 1995). Aside from these specific studies little is documented of dolphin immunocyte types especially lymphocyte subpopulations. Additionally, nothing has been reported on peripheral blood

immunocyte dynamics in health and disease or the potential effects of various environmental living conditions on immunocyte populations.

The West Indian manatee (Trichechus manatus latirostris) is listed as endangered in the United States with an approximate population of 1,800 animals (U.S. Fish and Wildlife Service, 1989; 1994). Since 1974, a well organized manatee carcass recovery program has existed to record, analyze and report causes of manatee mortality in the southeastern United States (Irvine, et al., 1981; Beck, et al., 1981).

Human-related factors have been by far the greatest identifiable cause of manatee deaths with annual mortality estimated at 10% of the total population (Van Meter, 1987). The largest single human-related mortality factor is collision with boats (Beck, et al., 1982; O'Shea, et al., 1985; Van Meter, 1987; U. S. Fish and Wildlife Service, 1989, 1994).

In addition, cold exposure has caused many deaths especially during the winters of 1977, 1981 and 1984 (O'Shea, et al., 1985; Caldwell and Caldwell, 1985). In January 1990, an unprecedented 45 manatees died in Florida following a period of freezing temperatures and an associated habitat loss of warm water effluent from electrical power plants (S.A. Wright, pers. commun.). In 1994, the overall manatee mortality was the second highest since 1974 with human-related and perinatal mortality categories increasing in overall numbers (Marine Mammal Commission, 1995).

The Miami Seaquarium has been authorized by the U.S. Fish and Wildlife Service to capture and rehabilitate diseased and/or injured manatees. At the Seaquarium the manatee rehabilitation program includes traumatic cases, cold exposure cases, and calf orphans. One

of the problems with manatee medical rehabilitation is that little baseline clinicopathologic data is available for this endangered species. An off line citation index performed of manatee biology indicates past studies of natural history (Husar, 1978; Ronald, et al., 1978; Hartman, 1979; Cardeihac, et al., 1984; Caldwell and Caldwell, 1985; Van Meter, 1987); anatomy (Fawcett, 1942a, 1942b; Quiring and Harlan, 1953; Ronald, et al., 1978; Piggins, Muntz, and Best, 1983; Van Meter, 1987); physiology (Scholander and Irving, 1941; Farmer, et al., 1979; Gallivan and Best, 1981; Gallivan, et al., 1983; Irvine, 1983; Burn, 1986; Burn and Odell, 1987); parasitology (Upton, et al., 1989); and pathology (Beurgelt, et al., 1984; Walsh, et al., 1987). White, et al. (1976), Medway and Black (1982), Medway, et al. (1982) and Bossart and Dierauf (1990) have published some of the first crude baseline hematologic, serum chemical, and blood coagulation studies on manatees. Converse, et al., (1994) recently published hematologic and serum chemistry values for Antillean manatees (Trichechus manatus manatus) in Guyana.

No in-depth studies have been published regarding immunocyte identification, including lymphocyte subpopulations in the manatee. Additionally, characterization of immunocyte dynamics in normal manatees versus those with illness or traumatic injuries and the potential effects of various environmental conditions on manatee immunocyte populations have not been investigated.

Immunity to disease involves a complex series of interrelated and mutually regulated events. Under optimum conditions this scheme functions to efficiently eliminate harmful organisms. In instances of disordered immunoregulation (e.g., immunosuppression

secondary to chronic disease, dolphin morbillivirus infection, neoplasia, "stress", etc.) the host is unable to mount an immune response sufficient to eliminate an invading microorganism resulting in infection and frequently a terminal septicemia.

Orchestrating the immune response involves the interaction between different immunocyte types and subpopulations of cells comprising the humoral and cell-mediated divisions of the immune system (Horton, 1985). The classic cell-mediated and humoral immune responses involve the interaction of numerous cell types including macrophages, T lymphocytes, and B lymphocytes (Reinherz and Schlossman, 1981; Green, 1983).

Activated macrophages engulf and process antigen and produce numerous cytokines (Thiele and Lipsky, 1982). These cytokines act as protein messengers and have numerous important functions including stimulation of T lymphocytes, some of which then coordinate the immune response while others destroy cells infected with intracellular organisms (Gupta, et al., 1987; Chen, 1990a).

T lymphocytes are categorized into three classes. T helper cells (T_H) act as the coordinators of the cell-mediated immune response (Benjamini and Leskowitz, 1988). In order for them to perform this function, they must communicate with other cells. This communication occurs through T helper cell production of specific cytokines called lymphokines. These lymphokines activate the other cells associated with the specific immune response (Chen, 1990a). For example, interleukin-2 produced by T helper lymphocytes is responsible for stimulating clonal expansion of T cytotoxic cells, B cells, and additional T helper cells (Tizard, 1986).

T cytotoxic lymphocytes (T_c) destroy antigenically-altered cells or cells containing intracellular organisms (Chen, 1990b). T cytotoxic cells are specific killer cells (in contrast to the non-specific killing activity associated with natural killer cells [NK cells]) (Tizard, 1992). T cytotoxic cells are important in controlling viral diseases and destroying neoplastic cells (Dinarello, 1988).

It is proposed that T suppressor lymphocytes (T_s) also produce lymphokines which are responsible for balancing the immune response and down-regulating the immune system once the disease situation is under control (Dinarello and Mier, 1987).

B lymphocytes can differentiate into plasma cells that manufacture antibodies capable of neutralizing specific extra-cellular organisms or labeling specific cells for immunodestruction (de Sousa, 1983). These humoral immune responses are generally slower than cell-mediated responses and are most important in systemic and/or bacterial diseases.

Natural killer cells (NK cells or large granular lymphocytes) are lymphoid cells found in the spleen, lymph nodes, bone marrow, and peripheral blood of non-immune rodents, domestic mammals and humans (Tizard, 1992). These cells can lyse a variety of target cells such as virally-infected cells, antibody-coated cells, undifferentiated cells, and some neoplastic cells (Benjamini and Leskowitz, 1988). The NK cells lack most of the characteristic cell surface markers of T cells, B cells, and macrophages. They are distinguishable from T cytotoxic cells since the activity of NK cells is not increased after immunization and is not dependent on the presence of antibody (Herberman, 1983). NK cell

activity can be augmented by exposure to bacterial adjuvants, tumor cells, virally-infected cells, and interferon, but the activation of NK cells does not involve immunologic memory (Lazarus and Baines, 1985). There is evidence to suggest that NK cells play a role in the host-tumor relationship and may be important in the host's defense against early tumor growth before the development of T cytotoxic cells and T cell-mediated activated macrophages (Herberman, 1983; Benjamini and Leskowitz, 1988; Roitt, 1988).

Over the past decade it has become apparent that peripheral blood lymphocytes constitute a heterogenous mixture of cells of different morphology and function representing different immunologic sub-populations which can now be identified with the use of specific monoclonal antibodies (Erber, et al., 1984; Jaffe, 1985; Sun, et al., 1985; Flynn, 1990; Butcher, 1990). In recent years, monoclonal antibodies have been used to determine the ratio of peripheral T helper lymphocytes to T cytotoxic/suppressor lymphocytes in human patients with acquired immune deficiency syndrome (AIDS). It has been reported that a decrease in the number of T helper cells is one of the earliest clinical events in the AIDS infection and this parameter can be utilized to predict when clinical AIDS will occur (Eyster, et al., 1987; Polk, et al., 1987).

These peripheral blood lymphocyte subset parameters utilizing monoclonal antibody methodology have also been determined in other human diseases and used as a diagnostic "window" into the function of the immune system. Diseases investigated in this manner include candidiasis, Down's syndrome, multiple sclerosis, pneumonia, Crohn's disease, pyelonephritis, Grave's disease, systemic lupus erythematosus, and pulmonary sarcoidosis

(de Sousa 1981, 1983; Gatenby, 1989; Morimoto, et al., 1987; Giorgi and Detels, 1989; Fike, 1990; Westermann and Pabst, 1990). Similar lymphocyte subset types have not been determined in dolphins and manatees, in either healthy or disease states, largely due to the unavailability of specific monoclonal antibodies (Cavagnolo, 1979; Romano, et al., 1992).

Recently, another method for lymphocyte subpopulation identification has been reported and validated in humans. This method involves the staining of fixed cells with an aqueous solution of a polymethine dye and viewing the stained specimen under darkfield illumination (Kass, 1988). This replaces complex monoclonal antibody techniques which are presently unavailable and economically impractical for use in non-laboratory animal species.

Understanding the interactions, events and dynamics that alter the numbers and functions of immunocytes is the first step in being able to identify animals which are likely to develop disease. In free-ranging species, this knowledge would widen the immunologic data base and might permit an assessment of population health before morbidity and mortality occur. It might also help to identify deleterious etiologic environmental factors such as anthropogenic toxins and infectious organisms which impact the immune system. These animals could then act as sentinel species for environmental health.

In captive animals, this knowledge could be beneficial for preventative husbandry and medical programs and might permit the development of immunologically-based treatment regimens.

The purpose of this study was to characterize immunocyte morphology in bottlenose dolphins and manatees utilizing traditional cytochemical techniques and the newly developed methodology of Kass (1988) for differentiation of lymphocyte sub-populations. Baseline peripheral blood immunocyte types and cellular distributions were determined in healthy animals living under various environmental conditions including free-ranging, stranded, and captive categories. Animals were also evaluated by age and sex. Cellular comparisons from peripheral blood and other lymphoid tissue compartments were made between healthy animals and those with documented clinical disease. Correlations were then made of immunocyte dynamics in healthy and disease states under various environmental living situations. Now, the functional implications of altered numbers of immunocytes in peripheral blood and other tissue compartments can be appreciated in these species and possibly, as in humans, be utilized as a diagnostic "window" in the immune system.

2.00 MATERIALS AND METHODS

2.10 STUDY POPULATION

Dolphins evaluated in this study included captive, free-ranging, and stranded animals (see Section 2.2). Facilities which provided samples from captive animals included Sea World (Florida, Texas, and Ohio parks), Ocean World (Ft. Lauderdale, Florida), Dolphin Research Center (Marathon, Florida), Ocean Reef (Key Largo, Florida), the United States Navy (Key West, Florida and Hawaii bases), the Indianapolis Zoo (Indianapolis, Indiana), and the Miami Seaquarium (Miami, Florida). Free-ranging dolphin samples were provided by Dr. Randall Wells of the Brookfield Zoo (Chicago, Illinois) as part of a long-term study of a discrete population of bottlenose dolphins in the Gulf of Mexico.

Stranded animal samples were collected under a Letter of Authorization from the National Marine Fisheries Service to Sea World of Florida, Dolphin Research Center, and the Miami Seaquarium at stranding sites along the southeast Florida coastline and the Florida Keys. All work on this project was approved by the FIU Animal Welfare and Use Committee.

A total of 180 dolphin blood samples were analyzed in this study which included 86 dolphins in various health categories (see Appendix A and Section 2.3).

Manatees evaluated in this study included captive and rescued animals from the State of Florida (see Section 2.2). Cooperating institutions included Sea World of Florida, Homosassa Springs State Park (Homosassa, Florida) and the Miami Seaquarium. Rescued manatees generally involved boat-related injuries (see Section 2.3) and were brought into

a cooperating oceanarium. Biologic specimens were obtained under a permit from the United States Fish and Wildlife Service.

A total of 56 manatee blood samples were analyzed in this study which included 29 manatees in various health categories (see Appendix A and Section 2.3).

2.20 ENVIRONMENTAL CATEGORIES

Dolphin and manatees were separated into four living environment status categories for statistical evaluation. The categories were captive-captured (Cc), captive-born (Cb), stranded/injured free-ranging (Fs), and free-ranging (Ff) animals. Cc animals (n=69 dolphin blood samples/n=2 manatee blood samples) were generally long-term captives (i.e., greater than five years in captivity) that were captured from a free-ranging state. Cb animals (n=53 dolphin blood samples/n=10 manatee blood samples) were born in captivity generally to Cc parents. Fs animals (n=40 dolphin blood samples/n=44 manatee blood samples) were beached dolphins (either singly or in a mass-stranding situation) or injured manatees generally from boat-related traumatic situations. Fs animals either died or were brought into captivity for treatment. The captive Fs animals were ultimately released back to a free-ranging state or transferred to a Cc category after 2 years in captivity. The Ff animals (n=18 dolphin blood samples) were free-ranging dolphins from Dr. Well's ongoing population study in the Gulf of Mexico (see Section 2.1). The Ff dolphins were net captured, biologic data and specimens collected, and released generally within 30 minutes. Largely because of the manatee's endangered status there were no animals in the Ff category.

2.30 HEALTH CATEGORIES

Animals were divided into four health categories based on clinical evaluation by an experienced marine mammal veterinarian. Clinical criteria for categorization included findings from physical examination and clinicopathologic data such as complete blood counts (CBC) and blood serum chemistries. Health categories included normal healthy animals (H1), animals with acute illness (H2), animals with chronic illness (H3), and animals with potentially altered physiologic states (H4).

H1 animals (n=53 dolphins/n=28 manatees) were clinically normal with no apparent disease conditions and had a normal total leukocyte count evaluation (see Section 2.6). Multiple blood samples from the same animal over a four year period were included in this study (n=5 dolphins/n=3 manatees).

H2 animals were acutely ill with an illness duration of under eight weeks and sub-categorized into H21 and H22 groups. H21 animals (n=56 dolphins/n=10 manatees) had elevated total leukocyte counts based on reference data established for the species (Bossart, 1990). These animals often had confirmed or suspected bacterial infections (as evidenced by clinical suspicion, microbiologic cultures and/or response to systemic antibiotic therapy). Multiple blood samples from the same animal during its illness were included in this study (n=13 dolphins/n=1 manatee) to help characterize immunocyte population dynamics in acute disease states and monitor clinical progress.

H22 animals (n=7 dolphins/n=1 manatee) had depressed total leukocyte counts based on the above reference data. These animals had clinical and pathologic signs consistent

with viral infection and/or a terminal septicemia. All the animals in the H22 subgroup died within 48 hours of blood sampling.

H3 animals (n=18 dolphins) had chronic clinical illness (e.g., pulmonary rales, cough, elevated hepatic serum enzymes, etc.) of greater than 8 weeks duration. Because of the time limitation, no free-ranging dolphins were present in this health category. The total leukocyte count could be normal or elevated in this category. This category in dolphins represented animals in a stable or a resolving infectious condition. Multiple blood samples from the same dolphin (n=5) were included in this category to help characterize immunocyte population dynamics in chronic disease states and monitor clinical progress. Interestingly, there were no manatees in the H3 category.

H4 animals (n=47 dolphins/n=7 manatees) included animals which had potentially altered physiologic states. This category included pregnant animals (n=9 dolphins/n=2 manatees), animals transported within a 48 hour period (n=36 dolphins/n=6 manatees), and 1 manatee suffering the physiologic effects of hypothermia ("cold-stress" syndrome).

2.40 AGE AND SEX CATEGORIES

The blood sample analyses were evaluated by sex and age for statistical comparisons. There were 67 male/113 female dolphin blood samples and 19 male/37 female manatee blood samples in this study. Age categories included animals less than or equal to 2 years of age (A1), animals from 2 to 10 years of age (A2), and animals over 10 years of age (A3).

In free-ranging animal environmental categories age was estimated by morphometric measurements and known natural history. The age category blood samples were broken

down as follows: A1 (n=7 dolphins/n=5 manatees); A2 (n=81 dolphins/n=26 manatees); and A3 (n=92 dolphins/n=25 manatees). Within the study period some individual animals had multiple blood samples taken including in A1 (n=1 dolphin), A2 (n=10 dolphins/n=3 manatees), and A3 (n=13 dolphins/n=2 manatees).

2.50 SAMPLE COLLECTION

2.51 PERIPHERAL BLOOD

Blood samples were collected from captive animals by two methods. One method involved draining water from the animal's habitat, placing the animal on a foam cushion, and then using manual restraint for the procedure. The restraint for this procedure generally required less than 15 minutes. The second method utilized a trained behavior. The animal was trained to present itself and hold for the entire procedure without the necessity of draining habitat water. With Ff dolphins, the animals were netted and after data collection were immediately returned to the Gulf of Mexico (generally less than 30 minutes).

In captive, clinically normal animals (H1 category) blood was taken as part of routine physical examinations. In clinically ill animals (H2 and H3) or H4 categories blood was generally taken to monitor clinical response to treatment or effects of physiologic conditions (e.g., pregnancy, etc.).

The ventral fluke or ventral peduncle (in dolphins) and ventral pectoral flipper (in manatees) were scrubbed for a 2 minute period with a 7.5% povidone-iodine surgical scrub preparation and rinsed with fresh water and then isopropyl alcohol. The anatomic locations for venipuncture were the periarterial venous plexus in dolphins and the interosseous vein or brachial plexus in manatees.

After aseptic site preparation, blood was taken using a 1.5 inch 20 gauge sterile needle and 12 ml sterile plastic syringe. Immediately following venipuncture, the blood was put in a 3 ml sterile interior glass tube containing an anticoagulant (0.06 ml of 7.5% of the

dipotassium salt of ethylenediaminetetraacetic acid [EDTA]). The tube was then gently rotated to insure thorough mixing of blood with anticoagulant. These specimens were kept cool at approximately 6° C and processed generally within 60 minutes from the time of collection.

2.52 LYMPHOID TISSUE

Lymphoid tissue (thymus, when present), (spleen and peripheral and mesenteric lymph nodes) were collected at necropsy from 15 dolphins and 10 manatees. Gross necropsies were performed by marine mammal veterinarians generally within 4 hours of death. Carcasses were kept cool with ice in the interim.

Lymphoid tissue specimens included touch preparations and formalin-fixed specimens. Touch preparations were prepared by gently touching a histology grade clean microscope slide to a cut section of lymphoid tissue. Generally four slides were made from each tissue type and allowed to air-dry prior to staining. Approximately 1 cm x 1 cm x 1 cm lymphoid tissue specimens for histopathologic evaluation were fixed in 10% neutral buffered formalin prior to processing. In addition to lymphoid tissues most necropsies included formalin-fixed tissue collection from all representative organs.

2.53 MURINE LYMPHOID TISSUE - POLYMETHINE STAIN VALIDATION STUDY

The differential coloration of lymphocyte subsets using the polymethine stain was validated in humans by Kass (1988) utilizing specific human monoclonal antibodies (mAb's) and cell sorting methodology. The demonstration of homologous staining of non-human

mammalian lymphocyte subsets using this stain and methodology was considered important to validate this procedure in dolphins and manatees where monoclonal antibodies (mAb's) are not available.

To this end, a validation study was designed using a mouse model. In this species, mAb's are commercially available to lymphocyte subset receptor molecules and are commonly used in immunologic studies.

Sorted mouse lymphocytes were provided by Garet Lahvis from the Department of Microbiology and Immunology of the University of Maryland at Baltimore School of Medicine. Twelve BALB/c female mice were purchased from Charles River Laboratories (BALB/cAnNCr1BR) (Wilmington, Massachusetts). All mice were housed in sterile micro-isolator cages (Lab Products, Inc., Mywood, New Jersey) in a restricted climate controlled animal room which was maintained on a 12 hour day/night cycle. Food and fresh water were provided daily ad libitum.

The mice were humanely euthanized. Splenic and thymic tissues were collected. Splenic lymphocytes were obtained by grinding spleens between opposing rough surfaces of sterile glass slides. These lymphocytes were suspended in a medium consisting of RPMI 1640 supplemented with 25mm HEPES (GIBCO BRL, Grand Island, New York) and 0.5% BSA (Sigma Chemical Co., St. Louis, Missouri). Thymocytes were prepared similarly.

T cell-enriched populations of splenic lymphocytes were isolated by passing cells through nylon wool (Wako Bio Products, Richmond, Virginia) using the protocol recommended by the manufacturer. Splenic lymphocytes which lacked CD8⁺ T cells were

prepared by suspending splenic lymphocytes in anti-CD8 (3.155) mAb (from the American Type Culture Collection, Rockville, Maryland), diluted in RPMI 1640, 25mm HEPES and 0.5% BSA.

Following a 30 minute incubation at room temperature in antibody, cells were incubated at 37° C for 40 minutes in complement. This process was then repeated. The cell preparations were then analyzed using fluorescence activated cell sorting (FACS) methodology. Staining for FACS involved incubation of cells with mAb's in phosphate buffered saline, supplemented with 2% fetal calf serum and 0.2% sodium azide. B lymphocytes were identified by positive staining with anti-kappa Ig antibody (Pharmagen, San Diego, California). T lymphocytes were identified with anti-Thy 1.2 (Becton-Dickinson, San Jose, California).

These antibodies were either directly conjugated with FITC (fluorescein isothiocyanate) or PE (phycoerythrin) or were conjugated to biotin and were secondarily stained with streptavidin-PE (Fisher Scientific, Pittsburgh, Pennsylvania) or streptavidin-FITC (Southern Biotechnology Associates, Inc., Birmingham, Alabama). Flow cytometry was conducted on a FACScan and analysis was conducted with the Consort 30 program (Becton-Dickinson, San Jose, California).

Sorted cell preparations included normal spleen cells, thymocytes, spleen cells depleted of T cytotoxic/suppressor lymphocytes and spleen cells depleted of B lymphocytes. Four air-dried smears of these cell preparations were prepared in an identical manner as the peripheral blood buffy coat smears and stained with the polymethine stain (see Section

2.62). FACS readings of the cell preparations indicated the percentage of cell types in each slide. These were then compared with the relative percentages of lymphocyte types from the polymethine-stained specimens for validation purposes.

2.60 SAMPLE ANALYSES

2.61 COMPLETE BLOOD COUNT WITH TRADITIONAL IMMUNOCYTE DIFFERENTIAL COUNTS FROM PERIPHERAL BLOOD

From the EDTA blood samples, hematologic red blood cell count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and total leukocyte (i.e., immunocyte, white blood cell [WBC]) counts were measured on a Coulter Counter Model S-Plus STKR in accordance with the manufacturer's manual (Coulter Electronics, P.O. Box 2145, Hialeah, Florida). The WBC differential counts (neutrophils, band neutrophils, total lymphocytes, monocytes, and eosinophils) were calculated from air-dried peripheral blood smears which were stained with a commercially available Wright-Giemsa preparation following manufacturer's instructions (Kacey, Inc., Miami, Florida). Differential cell counts were determined by counting 400 white blood cells under oil-immersion (1,000x) light microscopy and averaging these as 4 populations of 100 cells each. Relative differential counts were recorded as percent while absolute differential WBC counts numbers were calculated by taking the relative percentages of the total immunocyte count (see Appendix A). Statistical calculation utilized the absolute differential cell numbers.

2.62 POLYMETHINE-STAINED LYMPHOCYTE SUBSET DIFFERENTIAL CELL COUNTS FROM PERIPHERAL BLOOD

The presence and identification of lymphocyte subset populations (B cells, T helper cells, T cytotoxic/suppressor cells, and natural killer cells [NK cells]) were determined by utilizing the differential coloration produced by a newly developed commercially available polymethine dye p-ethoxy-phenyl-p-aminostyryl-1,3,3-trimethyl-3H-indolium chloride as an aqueous stain applied to white blood cell (buffy coat) peripheral blood smear preparations fixed in FAA fixative (95% ethyl alcohol 90 ml, 37% formalin 5 ml, glacial acetic acid 5 ml) (Lymphocolor, Cytocolor, Inc., Hinckley, Ohio). This stain as previously indicated was validated in humans for identification of lymphocyte sub-populations by Kass (1988).

Lymphocyte subsets were determined from buffy coat preparations of the EDTA blood samples. Buffy coat preparations were prepared by letting the plasma separate from the red blood cells by gravity in a vertically placed tube rack. Once separation had occurred, leukocyte-rich plasma was decanted into a sterile blank glass centrifuge tube and centrifuged at approximately 2500 rpm for 10 minutes. The plasma was decanted and the immunocyte button at the tube bottom was re-suspended in one-two drops of plasma.

This preparation was smeared on a clean cytologic grade microscope slide by gliding one drop over the slide surface by a spreader slide. This was accomplished by placing the spreader slide in front of the specimen drop at an angle of approximately 30° and pushing it back until it just touched the drop. As soon as the spreader slide touched the drop, the specimen spread along the slide edge by capillary action and the spreader was then

immediately pulled smoothly, quickly and gently at an angle of about 30°. These specimens were then allowed to air-dry. Four slides were prepared from each blood sample and were then stained with the polymethine dye preparation as detailed by the stain's manufacturer (Cytocolor, Inc., Hinckley, Ohio).

These stained buffy coat peripheral blood smears were then examined with darkfield microscopy using a Microstar IV microscope (Reichert-Jung, Cambridge Instruments Inc., Buffalo, New York) equipped with a 35 mm automatic camera system (Photostar, Reichert-Jung, Buffalo, New York). The darkfield technique utilized a darkfield oil condenser, 100 watt halogen light source, and a 100x oil immersion lens equipped with an iris diaphragm.

Immunocytes were identified by morphology and differential coloration as described by Kass (1988). In humans, neutrophils stained dark maroon and contained green granules, eosinophils contained bright blue cytoplasmic granules, and monocytes stained green with green and yellowish cytoplasmic vacuoles. In studies of purified lymphocyte subpopulations obtained in a cell sorter, T helper lymphocytes stained red, T suppressor cells were yellow-orange, B lymphocytes appeared yellow and frequently contained yellow annular cytoplasmic structures, and NK cells stained greenish-black with abundant, large green cytoplasmic granules. Based on the validation studies indicating homologous staining characteristics of immunocytes (see Section 3.3) this methodology was used in this study.

For each specimen, 400 lymphocyte subset cells were counted and then a mean calculated as a percentage of total circulating lymphocyte population. This, in essence, became a lymphocyte subset relative differential count which expanded the standard

immunocyte relative differential count determined in Section 2.61. Absolute numbers of each circulating lymphocyte subset (T helper, T cytotoxic/suppressor, B cell, and NK cell) were determined by taking each cell's percentage of the total circulating lymphocyte count determined in Section 2.61. Absolute numbers of cellular subsets were used in statistical comparisons.

2.63 POLYMETHINE-STAINED LYMPHOID TISSUE PREPARATIONS

Touch preparations of primary and secondary lymphoid tissue from Section 2.52 were stained with the polymethine stain. The staining procedure was identical to that used for the peripheral buffy coat smears from Section 2.62. In that section, a subjective evaluation of lymphocyte subset types, relative cell numbers and distributions was proposed to compare with the peripheral blood and histopathologic findings from the same tissues.

Using this technique, characterizations of immunocyte population shifts could be made between tissue compartments. For example, if histopathologic evidence of lymphoid depletion was present in a case, a correlation was made with lymphocyte subset populations in terms of cell types, numbers, etc. from the polymethine-stained preparations from the same case. Alternatively, if lymphoid hyperplasia with an architectural pattern suggesting B and T cell components was present, a similar correlation was made with lymphocyte subset populations from the polymethine-stained tissues from that same case. This approach was selected because experimental trials indicated that the polymethine selective lymphocyte staining did not function in paraffin-embedded formalin-fixed tissue specimens.

Lymphocyte sub-populations from lymphoid tissue were evaluated in an identical manner as the peripheral blood buffy coat smears (see Section 2.62).

2.64 HISTOPATHOLOGIC EVALUATION OF LYMPHOID TISSUE

Formalin-fixed lymphoid tissues collected in Section 2.52 were processed in increasing grades of methyl alcohol followed by xylene and then embedded in paraffin. Paraffin tissue blocks were sectioned on a microtome at approximately 5 microns and stained with hematoxylin and eosin. Specimens were examined using the same microscopic equipment as in Section 2.62. In selected cases other histochemical stains were occasionally used to diagnose etiologic agents including Brown and Brenn, Grocott methenamine silver and periodic acid-Schiff.

2.70 STATISTICAL EVALUATIONS

Statistical analyses were made using J.M.P. software (S.A.S. Institute, Cary, North Carolina) on a Macintosh computer Model IIVx (Apple Computers, Itasca, Illinois). The Tukey-Kramer HSD (honesty significant difference) test was used for comparisons for all pairs of means. The Tukey-Kramer HSD test is sized for all differences among the means and is an exact alpha-level test if the sample sizes are the same, and conservative if the sample sizes are different. Comparisons of immunocyte means were determined among health, environmental-living, age and sex categories. The software program also generated a visual comparison of group means. A P value of ≤ 0.001 was considered significant unless otherwise indicated in the text. Tables of significantly different immunocyte parameters are found in Appendix B.

3.00 RESULTS

3.10 DOLPHIN IMMUNOCYTE MORPHOLOGY - WRIGHT-GIEMSA STAINED PERIPHERAL BLOOD

With Wright-Giemsa stained peripheral blood specimens neutrophils, total lymphocytes, eosinophils, and monocytes were identified in most cases. The most common circulating immunocytes in clinically normal animals were neutrophils followed by, in decreasing frequency, usually lymphocytes, eosinophils, monocytes, and band cells. Basophils were not identified in the study.

Neutrophils were typically mammalian and were the most common immunocyte in the peripheral blood of dolphins. Neutrophils ranged from 10-15 μm in diameter and were characterized by an irregularly lobed nucleus with the occasional formation of fine filaments connecting the lobes. The nuclear membrane was often irregular. The cytoplasm stained a faint pinkish-gray with granules appearing as fine, discrete particles and occasional well-defined cytoplasmic vacuoles.

The band neutrophil was rarely seen in the peripheral blood of dolphins. This cell was approximately 15 μm in diameter with a nucleus characterized by a curved band with a smooth nuclear membrane. The sides of the nucleus were parallel with less dense nuclear chromatin. The cytoplasm was pale gray with pale-staining neutrophilic granules.

The eosinophil was approximately the same size as the neutrophil and had a segmented nucleus similar to the mature neutrophil. The cytoplasm contained usually

densely packed pink granules which varied little in size, shape, and staining characteristics. In some eosinophils these granules partially or completely covered the nucleus.

Total lymphocytes were represented by two types based on relative size. The predominant population was the small type measuring approximately 8-10 μm in diameter with a narrow rim of slightly basophilic cytoplasm and an eccentrically located nucleus containing clumped chromatin that stained intensely basophilic (Plate 3.101). The large type of lymphocyte measured up to 16 μm in diameter and occurred with less frequency but had a similar morphology. Rarely, large lymphocytes contained azurophilic cytoplasmic granules.

The monocyte was generally the largest cell in the peripheral blood, measuring from 12-17 μm in diameter. The cytoplasm was basophilic and had a pale blue, ground-glass appearance. Cytoplasmic vacuoles were frequently present giving a foamy appearance. The nucleus was markedly pleomorphic in size and shape. Nuclear folds were common and the nuclear chromatin pattern was characteristically diffuse and lacy.

Plate 3.101 - Dolphin lymphocytes (tailless arrows), plasma cell (arrowhead), and macrophage (tailed arrow). Wright-Giemsa. Bar=10 μ m.

3.20 MANATEE IMMUNOCYTE MORPHOLOGY - WRIGHT-GIEMSA STAINED PERIPHERAL BLOOD

With Wright-Giemsa stained peripheral blood smears from manatees, heterophilic granulocytes (i.e., neutrophils), band cells, total lymphocytes, eosinophils, and monocytes were identified in most cases. However, the predominant circulating cell types differed from dolphins. The most common circulating leukocytes in clinically normal manatees were heterophilic granulocytes followed by in decreasing frequency, lymphocytes, monocytes, and eosinophils and band cells. As with dolphins, basophils were not identified in manatees.

A unique characteristic of the manatee polymorphonuclear granulocyte was that with Wright-Giemsa stained specimens it was heterophilic-like rather than neutrophilic. The segmented heterophilic granulocyte was the most common leukocyte in the peripheral blood. These round cells were similar in size with other mammalian neutrophils ranging from 10-15 μm in diameter. The nucleus, as in dolphins, was multilobulated with usually coarse clumped dark blue to purple staining chromatin. The nuclear membrane was sometimes irregular. The cytoplasm was generally colorless and contained densely packed, pleomorphic, fine eosinophilic granules. The nucleus was often partially hidden by these cytoplasmic granules (see Plates 3.201 and 3.202).

Band heterophilic granulocytes were very rare circulating leukocytes in clinically normal manatees. These cells had similar nuclear characteristics as dolphin band cells. The cytoplasm, however, contained eosinophilic granules similar to the mature heterophilic granulocyte.

Manatee lymphocytes were the second most common circulating leukocyte in clinically normal animals and were present in higher relative and absolute numbers compared with dolphins (mean 40% vs. 20% respectively, see Table 3.531). Small type lymphocytes were most common and were similar in morphology to the small lymphocytes observed in dolphins. Occasional large lymphocytes contained granular basophilic cytoplasm.

Eosinophils were approximately the same size as the dolphin eosinophil but were present in smaller relative and absolute numbers (mean 5% vs. 15% in dolphins, see Table 3.531). Eosinophil nuclei were hyposegmented with coarse basophilic chromatin. The cytoplasm was clear and pale blue and contained generally monomorphic, round, non-refractile eosinophilic granules that were very distinct and individualized helping to differentiate them from heterophilic granulocytes (Plate 3.202). Eosinophilic granules frequently had different staining qualities from heterophilic granules in the same blood smear. Granules would appear either duller or brighter pink compared with heterophilic granules.

Manatee monocytes were typical mammalian in size and morphology and similar to dolphin monocytes (Plate 3.201).

Plate 3.201 - Manatee heterophilic granulocytes with Barr bodies (arrowhead) and monocyte (arrow). Wright-Giemsa. Bar=10 μ m.

Plate 3.202 - Manatee heterophilic granulocytes and degranulated eosinophil (arrow).

Wright-Giemsa. Bar=10 μ m.

3.30 MURINE LYMPHOID TISSUE - POLYMETHINE STAIN VALIDATION STUDY

FACS readings of non-depleted spleen preparations revealed a mean lymphocyte population composed of 86% B lymphocytes and 14% T lymphocytes (11% T helper lymphocytes and 3% T cytotoxic/suppressor lymphocytes). Polymethine-stained specimens from this preparation revealed cells consistent with Kass' classification as 80% B lymphocytes, 19% T lymphocytes (15% T helper cells and 4% T cytotoxic/suppressor cells) and 1% natural killer cells.

FACS readings of T cytotoxic/suppressor depleted spleen preparations revealed a lymphocyte population of 87% B lymphocytes and 12% T helper cells and less than 0.1% T cytotoxic/suppressor cells. Polymethine stained slides from this preparation revealed by the Kass classification 90% B lymphocytes, 9% T helper cells and 1% natural killer cells.

FACS readings of B cell depleted spleen preparations revealed a lymphocyte population of 20% B lymphocytes and 80% T cells. Polymethine stained slides evaluated with Kass' criteria revealed 12% B lymphocytes and 87% T lymphocytes (69% T helper cells and 18% T cytotoxic/suppressor cells), and 1% natural killer cells.

In addition, murine thymocyte (T lymphocytes) preparations were stained with the polymethine stain. By Kass' cytologic criteria this preparation contained 90% T helper cells and 10% T cytotoxic/suppressor cells. No B lymphocytes were identified in this preparation. These results validated this stain in the mouse according to the human classifications for

lymphocyte subset differentiation (Kass, 1988). Homologous staining was then inferred for other mammals including dolphins and manatees.

3.40 DARKFIELD MICROSCOPIC EXAMINATION OF POLYMETHINE STAINED PERIPHERAL BLOOD PREPARATIONS FROM DOLPHINS AND MANATEES

Similar to the findings with human and mouse immunocytes using the polymethine dye, differential coloration of dolphin and manatee immunocytes was achieved using darkfield illumination. Immunocytes displayed various shades of pink under conventional white light illumination. Under darkfield illumination, however, the same cells exhibited a variety of different colors against a black background. Under darkfield illumination, the cells had bright often startling colors and were easily visualized once the darkfield oil iris methodology was mastered. Differential staining characteristics permitted identification of neutrophils (in dolphins), heterophilic granulocytes (in manatees), eosinophils, and monocytes based on typical mammalian morphologic characteristics. T helper lymphocytes, T cytotoxic/suppressor lymphocytes, B lymphocytes, and natural killer (NK) cells were identified based on differential coloration reported by Kass. In all cells, details of the nuclei and cytoplasm were unusually precise with this stain when viewed under darkfield illumination.

Neutrophils, heterophilic granulocytes, eosinophils, and monocytes were found in approximately the same relative numbers with this stain compared to the Wright-Giemsa stain. Neutrophils and heterophilic granulocytes contained dark green-brown nuclei with

identical morphology to those stained with Wright-Giemsa. Dolphin neutrophil cytoplasm, as in humans, was dark maroon and contained many dark green-black granules. On the other hand, manatee heterophilic granulocyte cytoplasm was dark black with abundant bright green, blue, red, and sometimes white pleomorphic granules.

Eosinophils in both dolphins and manatees showed identical nuclear morphology as those in Wright-Giemsa stained specimens. The cytoplasmic granules with the polymethine stain were bright turquoise-blue which is similar to human eosinophil granules described by Kass (see Plate 3.401).

Monocytes were characterized in both groups by similar morphology. The nuclei and cytoplasm stained pale green with nuclei having typical convolutions and indentations. In some monocytes, yellow staining vacuoles were present.

Dolphin and manatee lymphocytes revealed a variety of shapes and colors similar to known purified lymphocyte sub-populations in humans and mice. The majority of lymphocytes were small and had cytoplasm that appeared roughened and stained bright red. The nuclei in these lymphocytes stained red to black, with occasional bright red strands. By the Kass classification, these cells were characteristic of T helper lymphocytes found in humans and mice (see Plates 3.403 and 3.405). These cells were the predominant circulating lymphocyte in clinically normal dolphins and manatees representing between 75%-89% of total circulating lymphocytes. As previously indicated manatees had higher relative/absolute numbers of circulating total lymphocytes which were reflected in lymphocyte subset populations absolute numbers.

A second type of lymphocyte was identified in lesser numbers than the T helper cells. These cells were for the most part slightly larger than T helper lymphocytes and were characterized by smooth, bright yellow cytoplasm with black dense granular nuclei interspersed with linear strands and clumps of yellow. These lymphocytes corresponded to T cytotoxic/suppressor cells as described by Kass (1988) and characterized in the mouse validation study (Plates 3.401, 3.403 and 3.405).

Lymphocytes of the fewest numbers in clinically normal animals were of two types. The first was a cell slightly larger than the T cytotoxic/suppressor lymphocyte. The cytoplasm was more abundant with a smoothly black background and contained small varying numbers of bright yellow annular structures. The nucleus was smooth and stained dull black with rare strands of yellow. This cell type had staining characteristics similar to the B lymphocyte identified by Kass and seen in the mouse validation study (Plate 3.402). The second type was the largest lymphocyte observed and usually the rarest in clinically normal animals. This lymphocyte had abundant greenish-black smooth cytoplasm containing only occasional to densely packed small pleomorphic round green granules. The round nucleus was smooth and stained black and occasionally had green chromatin strands. This putative NK cell had characteristics consistent with the human natural killer (NK) cell identified by Kass (1988) and were approximately 0-2% of circulating lymphocytes (Plate 3.404). In addition, in these specimens erythrocytes stained orange-red and platelets stained dull maroon with occasional green granular intracellular structures.

Plate 3.401 - Dolphin eosinophils (small arrows), T_{cs} cell (arrowhead), and monocyte (large arrow). Polymethine stain. Bar=10 μ m.

Plate 3.402 - A dolphin B cell (arrow). Polymethine stain. Bar=10 μ m.

Plate 3.403 - Dolphin T_h (large arrowhead) and T_{cs} (small arrowhead) cells. Polymethine stain. Bar= $10\mu\text{m}$.

Plate 3.404 - A manatee NK cell (arrow). Polymethine stain. Bar=10 μ m.

Plate 3.405 - Manatee T_h (large arrowheads) and Tc/s (small arrowheads) cells.
Polymethine stain. Bar= $10\mu m$.

3.50 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS

3.51 HEALTH AND ENVIRONMENTAL CATEGORY IMMUNOCYTE DYNAMICS -DOLPHINS

Total mean white blood cell (WBC) counts in clinically normal (H1) dolphins ranged from 5,111-9,187 cells/mm³ in the designated living environment categories (Table 3.511). The free-ranging (Ff) dolphin group had significantly higher mean total WBC counts (9,187 cells/mm³) than the other 3 environmental groups which was due to higher numbers of circulating eosinophils. No significant differences in mean total WBC counts were present between the other environmental categories.

Neutrophils ranged from 3,379-4,260 cells/mm³ in the environmental categories comprising from 50-67% of the circulating immunocytes in clinically normal dolphins. No significant differences were present in absolute circulating neutrophil numbers between the 4 environmental categories.

In the H1 dolphin group, lymphocytes made up the second most predominant leukocyte ranging from 19-22% of circulating leukocytes in the 4 environmental categories. Absolute lymphocyte numbers ranged from 1,049-2,030 cells/mm³. The Ff group had significantly higher mean absolute lymphocyte numbers (2,030 cells/mm³) compared with the other categories. No significant differences in lymphocytes were present between the other 3 environmental groups.

In H1 dolphins, all lymphocyte subsets were found in each group in decreasing order of frequency (relative/absolute numbers) as follows: T helper lymphocytes (T_h) (73-89%, 765 - 1,814 cells/mm³), T cytotoxic/suppressor lymphocytes (T_{cs}) (8-21%, 137-239 cells/mm³), B lymphocytes (1-6%, 25-75 cells/mm³) and NK cells (1-4%, 6-46 cells/mm³). The Ff group had significantly higher mean numbers of T helper lymphocytes (1,814 cells/mm³). No significant differences were present between other lymphocyte subsets in the remaining environmental categories.

In H1 dolphins, eosinophils were found in all groups and were the third most numerous circulating leukocyte ranging from 7-29% (364-2641 cells/mm³). The Ff group had markedly and significantly higher absolute numbers of eosinophils compared with the other groups. Also the Cc group had significantly higher mean absolute eosinophils compared to the Fs group.

Monocytes were the least numerous cells in H1 dolphins ranging from 2-6% (125-313 cells/mm³) of the circulating leukocytes. The Cb group had significantly less absolute mean monocyte numbers than the Ff and Fs groups (125 cells/mm³ versus 273 and 313 cells/mm³, respectively).

T helper to T cytotoxic-suppressor lymphocyte ratios ($T_h:T_{cs}$) were calculated for clinically normal dolphins in each living environment category and ranged from 3.4 to 10.7.

The low end of the range included Cc and Fs categories (4 and 3.4, respectively). The Ff categories had a significantly higher ratio (10.7) compared with the other categories which was a result of higher numbers of circulating T_h lymphocytes.

Total mean WBC counts in acutely ill dolphins with probable bacterial infections (H21 group) ranged from 12,144 to 15,153 cells/mm³ in the designated living environment categories (Table 3.512). All immunocytes characterized in the H-1 category were present in this category, however significant differences occurred in cellular proportions. In Cb, Cc, Fs and Ff categories, WBC counts were significantly higher in the H21 group compared to the healthy dolphin group. In Cb, Cc, Fs, and Ff categories these elevated WBC counts were due to significantly higher circulating neutrophils and eosinophils. Total lymphocytes were significantly elevated in Cc and Cb categories and monocytes in Cb, Cc and Ff categories. Of the lymphocyte subsets, significant elevation occurred in T_h cells (in Fs), T_{e,s} cells (in Cb), and B lymphocytes (in Ff) compared to the healthy dolphin group. The remaining lymphocyte subsets were not significantly affected by this acutely ill probable bacteremic state.

With this acutely ill group, comparisons were made between living environment status (Table 3.512). Of all immunocytes including lymphocyte subsets, the eosinophil was the only immunocyte which was significantly different in absolute numbers. In the Ff group eosinophils were significantly elevated which follows the trend of higher absolute eosinophil numbers in the clinically normal dolphin Ff group.

The T_h:T_{e,s} ratios were calculated for acutely ill dolphins with probable bacterial infections in each living environment category and ranged from 2.8 to 13.8. This ratio dropped dramatically for Cb ill dolphins which was largely due to increased numbers of T_{e,s} lymphocytes. Alternatively, this ratio increased slightly in Fs and Ff ill dolphins due

primarily to relative increases in T_h lymphocytes. In Cc ill dolphins, the $T_h:T_c/s$ ratio remained the same as the healthy dolphin group.

Total mean WBC counts in acutely ill dolphins with terminal septicemia and/or viral infections (H22 group) ranged from 2,825-3,000 cells/mm³ and were represented only in the Cc (n=5) and Fs (n=2) living environment categories (Table 3.513). All of the dolphins in this group died within 48 hours of blood sampling. In the Fs group, all immunocytes characterized in the previous health categories were seen except B lymphocytes and NK cells. In this health group total mean WBC counts were significantly lower compared with the acutely ill dolphins of the H21 group in the Cc living environment category. Following this trend, significantly lower absolute mean numbers of neutrophils, total lymphocytes, eosinophils and monocytes were present as were lower cell numbers in T_h , $T_{c,s}$, and B lymphocyte subsets. No significant changes in immunocytes were present between the H22 group and the clinically normal dolphins. The Fs category in this health group was not evaluated due to insufficient sample size.

The $T_h:T_{c,s}$ ratios were markedly depressed in the H22 group compared to the healthy group and acutely ill group with probable bacterial infections (Table 3.513). In both the Cc and Fs categories this was due to combined depression of T_h lymphocytes and elevation of $T_{c,s}$ lymphocytes.

In chronically ill dolphins (H3 group) the total mean WBC counts ranged from 9,890-10,675 cells/mm³ and were represented by only 2 living environment categories (Cb and Cc) (Table 3.514). Compared with clinically normal dolphins, the total mean WBC

counts were significantly higher in these categories because of different immunocyte proportions. The WBC elevation in the Cb category was due to significantly higher numbers of neutrophils while in the Cc category it was a result of significantly more eosinophils. No other significant differences were found in immunocyte dynamics between the H3 group and clinically normal group in these living environment categories.

Compared with acutely ill dolphins, the chronically ill dolphins had altered immunocyte dynamics. Total mean WBC counts were significantly lower in both Cb and Cc categories. In Cb dolphins this was due to significantly fewer total lymphocytes and monocytes while in Cc dolphins it was due to significantly fewer neutrophils exclusively. No significant changes were noted in lymphocyte subset dynamics.

The $T_h:T_{cs}$ ratios for these chronically ill dolphins were approximately the same as clinically normal dolphins for both environmental categories (Table 3.514).

Total mean WBC counts in dolphins with potentially altered physiologic states (H4 group) ranged from 5,683-9,014 cells/mm³ and were represented in all environmental categories. Immunocytes of all cell types were identified and were not significantly different than those reported in the clinically normal dolphin group (Table 3.515).

3.52 AGE AND SEX IMMUNOCYTE DYNAMICS - DOLPHINS

All immunocyte types were found in clinically normal male and female dolphins (Table 3.521). Except for monocytes, no significant differences in immunocytes were found between clinically normal male and female dolphins. Males had significantly higher

circulating monocytes (mean of 279 cells/mm³, n=27 versus 186 cells/mm³ for females, n=26).

Except for eosinophils in acutely ill dolphins (H21 group), no significant immunocyte sex differences were found in both the acute and chronic (H3 group) illness groups. In H21 dolphins, males had significantly higher eosinophil numbers (2,565 cells/mm³ versus 1,318 cells/mm³ for females).

Female dolphins in the H4 group had significantly higher mean WBC counts and neutrophils compared to H4 males (6,770 and 4,060 cells/mm³ versus 5,780 and 3,418 cells/mm³, respectively). All other immunocyte parameters were not significant when compared between male and female dolphins.

In clinically normal dolphins in the three specified age groups (A1, A2 and A3) no significant differences were present in total WBC counts, neutrophils, total lymphocytes, T_h lymphocytes, T_c, lymphocytes, B lymphocytes, NK cells, eosinophils, and monocytes (Table 3.522).

Likewise in acutely ill dolphins with leukocytosis (H21) and leukopenia (H22) no significant immunocyte differences were found in animals within these age categories. In chronically ill dolphins (H3) however, significant differences occurred in total lymphocytes, B lymphocytes and NK cells between A2 and A3 animals (no A1 dolphins were represented). In H3 dolphins between 2 and 10 years of age (A2), total lymphocytes were significantly higher compared to dolphins over 10 years of age (A3). This elevation of total lymphocytes was due exclusively to significantly higher circulating numbers of B

lymphocytes and NK cells. Alternatively, the A3 dolphins in this health category had total lymphocytes, B lymphocyte, and NK cell levels which were quantitatively similar to the clinically normal dolphin group.

In the dolphins under physiologic altered conditions (H4), significant cellular differences between A2 and A3 age groups were present in total WBC counts, neutrophils and eosinophils. In the A1 group no statistical evaluation could be made because of the small sample size (n=1). In H4 dolphins between 2-10 years of age (A2), total WBC counts were significantly lower than in dolphins over 10 years of age (A3) which was entirely due to significant decreases in neutrophils and eosinophils. In this instance, as well, the A3 age group was similar to the clinically normal dolphin group in these cellular parameters.

3.53 HEALTH AND ENVIRONMENTAL CATEGORY IMMUNOCYTE DYNAMICS -MANATEES

Total mean WBC counts in clinically normal manatees (H1 group) ranged from 7,300-10,905 cells/mm³ in Cb, Cc, and Fs living environment categories (Table 3.531). Statistical evaluations were not performed for the Cc manatee category because of the small sample size (n=2).

Immunocytes of all types were characterized in the H1 manatee group and no significant differences were found between immunocyte types in captive born (Cb) and free-ranging injured (Fs) manatees.

Heterophilic granulocytes ranged from 5,021-6,132 cells/mm³ in Cb and Fs categories in clinically normal manatees comprising from 52-56% of the total circulating leukocytes.

Total lymphocytes in the H1 group ranged from 4,256-4,290 cells/mm³ comprising 39-44% of the total circulating leukocytes in both environmental categories. All lymphocyte subsets were identified in these categories. Total lymphocytes included 84-89% T_h lymphocytes (3,593-3,805 cells/mm³), 7-11% T_{c/s} lymphocytes (312-471 cells/mm³), 2-3% B lymphocytes (89-116 cells/mm³), and 2% NK cells (76-83 cells/mm³).

In H1 manatees, monocytes and eosinophils were found in 3-4% (378-442 cells/mm³) and 1% (17-66 cells/mm³) respectively in both represented living environment categories. T_h:T_{c/s} ratios ranged from 7.6-12.2 in clinically normal manatees in the Cb and Fs categories.

Total mean WBC counts in acutely ill manatees with probable bacterial infections (H21 group) ranged from 15,300-17,713 cells/mm³ in only the Cb and Fs living environment categories (Table 3.532). Statistical evaluations were not performed because of the sample size of the Cb category (n=1). All immunocytes found in the H1 group were present in this health category and the total mean WBC counts were significantly higher than those of the clinically normal manatee group. These higher WBC counts were reflected in significantly higher mean numbers of heterophilic granulocytes, total lymphocytes, T_h lymphocytes, T_{c/s} lymphocytes, NK cells, and monocytes.

The $T_h:T_c$ ratio of the H21 group was lower than both healthy environment groups at 5.5 (vs. 7.6 for Cb and 12.2 for Fs). This was due primarily to disproportional increases in T_c lymphocytes (Table 3.532).

Only one manatee (Fs) was represented in the acutely ill health group with terminal septicemia and a viral infection (H22) (Table 3.533). Therefore statistical evaluations could not be made for this category.

The total mean WBC count for Fs manatees under potentially altered physiologic states (H4) was 9,474 cells/mm³ and was not significantly different than the clinically normal manatee group. In the H4 manatee group, only Fs animals were represented for statistical evaluation (Table 3.534). While total mean WBC counts were not significantly different, significant differences were present in immunocyte types. In H4 manatees, heterophilic granulocytes were significantly elevated compared with the clinically normal group (7,062 vs. 5,021 cells/mm³, respectively). On the other hand, total lymphocytes and T_h lymphocytes were significantly lower than the clinically normal group (2,017 and 1,640 cells/mm³ vs. 4,290 and 3,805 cells/mm³, respectively). No other significant differences were present between immunocytes in this category.

The ratio for this H4 group was 6.6 which was lower than clinically normal manatees of the same environmental living status. This was due to a decreased number of T_h lymphocytes.

3.54 AGE AND SEX IMMUNOCYTE DYNAMICS-MANATEE

The total WBC count means for clinically normal (H1) male and female manatees were 10,804 cells/mm³ and 9,276 cells/mm³ respectively. They were not significantly different. No significant differences in any immunocyte types were present between males and females in the H1, H21 and H4 groups (Table 3.541).

Statistical evaluation of normal immunocyte means could be made for manatee age groups (Table 3.542) between 2 and 10 years of age (A2) and over 10 years of age (A3) only since in the manatee age group less than or equal to 2 years of age (A1) only 2 individuals were represented. In the clinically normal manatee health category, B lymphocytes were significantly higher in the A3 age group compared with the A2 group. No significant differences were present between any other immunocyte types.

Acutely ill manatees (H21) included 7 animals in the A2 age group. Statistical evaluations between groups A1 and A3 could not be accurately determined due to a low sample size of n=1 and n=2, respectively.

Only 1 manatee was present in the H22 acute health category, and none in the chronically ill health category, therefore no age comparisons could be made.

Likewise, manatees under physiologic altered conditions (H4) included 16 animals but 13 of these were in the A3 age group. The A1 and A2 age groups had 2 and 1 manatees respectively which precluded statistical evaluations in these categories.

3.55 IMMUNOCYTE DYNAMICS - INDIVIDUAL SERIAL CASE STUDIES

Serial blood sample data on the same individual dolphins (n=10) and manatees (n=4) over designated time periods are presented in Tables 3.551 and 3.552, respectively. Dolphins were represented in either the Cb or Cc environmental group, but in different and/or shifting health categories as follows, 1) a clinically normal (H1) dolphin (for comparative purposes), 2) acutely-ill (H21) dolphins (n=4), 3) chronically-ill (H3) dolphins (n=1), and 4) dolphins which converted from H21 to H3 over time (n=4). The manatees were represented in the Fs environmental group as follows, 1) a clinically normal (H1) manatee (for comparative purposes), 2) an acutely-ill (H21) manatee, 3) a pregnant (H4) manatee, and 4) a "cold-stressed" (H4) manatee.

The immunocyte dynamics of each animal over time are presented due to the generally unique clinicopathologic characteristics of each case. Only animals sampled 3 or more times are presented in this section for better clarity of immunocyte trends (see Tables 3.551 and 3.552).

3.60 LYMPHOID TISSUE EVALUATION

3.61 POLYMETHINE STAINED TOUCH PREPARATIONS

Touch preparations of lymphoid tissue from both manatees and dolphins were difficult or impossible to evaluate microscopically with the polymethine stain. This was due primarily to the thickness of touch preparations. Specimens were consistently too thick for accurate cellular differentiation which precluded any objective evaluation of lymphocyte

subset types, relative cell numbers and/or anatomic cellular distributions. In many specimens cellular artifacts and debris were also present.

Of the 15 dolphin and 10 manatee touch preparations, 4 dolphin and no manatee specimens were adequate for cellular differentiation. In the usable dolphin specimens T_h lymphocytes, T_c lymphocytes and B lymphocytes could be identified with difficulty only along the peripheral margin of the touch preparations. This was generally the thinnest portion of the specimen and provided the most contrast for cellular differentiation. No further meaningful interpretations could be made from these specimens.

3.62 HISTOPATHOLOGIC EVALUATION OF LYMPHOID TISSUES

Secondary lymphoid tissue including peripheral and mesenteric lymph nodes (pseudopancreas) and splenic tissues were examined microscopically from 15 dolphins and 10 manatees at necropsy (Table 3.621).

All of the dolphins examined had multi-systemic illness that in most cases resulted in death (Table 3.622). Ten had pneumonia (primary viral etiology with secondary bacterial and/or fungal opportunistic infections), 1 had suspected viral hepatitis, 3 had enterocolitis, and 1 had meningitis. All infections were of a chronic or chronic-active nature.

In all cases of pneumonia and the hepatitis case, severe lymphoid depletion occurred in the lymph nodes (Plate 3.622). The depletion occurred in both follicular (B cell dependent zone) and paracortical (T cell dependent zone) areas (Table 3.621).

In nine cases of pneumonia and the hepatitis case, moderate to severe lymphoid depletion also occurred in the spleens. This lymphoid depletion included germinal centers

(B cell dependent zone) and periarteriolar lymphoid sheaths (T cell dependent zone) (Table 3.621). The enterocolitis cases, 1 pneumonia case, and the meningitis case had no splenic lymphoid depletion. Alternatively, splenic lymphoid hyperplasia was present in the meningitis case, 1 pneumonia case and 1 enterocolitis case. One enterocolitis case also had gut-associated (GALT) lymphoid hyperplasia (Table 3.622).

All of the manatees examined had been killed as a result of human-related factors such as boat collisions and trauma was the primary cause of death. None of these animals had significant systemic illness based on gross and histopathologic analyses which could have resulted in death by itself. Unlike the dolphins, only 1 manatee had mild T cell dependent lymph node depletion and 1 case had combined mild splenic lymphoid depletion limited to B cell dependent zones only and moderate plasma cell hyperplasia (Table 3.621). Six manatees had mild to moderate lymph node lymphoid hyperplasia (Plates 3.624 and 3.625) which was in marked contrast to the dolphin group (Table 3.623). Four had combined B and T cell dependent zone hyperplasia, 1 had B cell dependent zone hyperplasia only and 1 had medullary plasma cell hyperplasia (Table 3.623).

Plate 3.621 - A normal dolphin lymph node. Notice prominent lymphoid follicles (H) and paracortical areas (T). H&E. Bar=100 μ m.

Plate 3.622 - A dolphin lymph node with severe follicular and paracortical lymphoid depletion. H&E. Bar=50 μ m.

Plate 3.623 - A lymph node with pronounced lymphoid depletion from a morbillivirus-infected dolphin. Few lymphoid areas remain which highlights supporting collagenous tissue stroma. H&E. Bar=100 μ m.

Plate 3.624 - A manatee lymph node characterized by marked lymphoid hyperplasia.

H&E. Bar=100 μ m.

Plate 3.625 - A higher power photomicrograph of plate 3.624 showing follicular lymphoid hyperplasia with mitotic figures. H&E. Bar= $10\mu\text{m}$.

4.00 DISCUSSION

4.10 IMMUNOCYTE CHARACTERIZATION

4.11 MORPHOLOGY AND VALUES OF IMMUNOCYTES IN HEALTHY DOLPHINS

Total mean WBC counts and standard differential immunocyte values for clinically normal dolphins were comparable to terrestrial domestic mammalian herbivores and carnivores with some notable exceptions. Additionally, immunocyte morphology with Wright-Giemsa stained specimens was similar to other domestic mammalian herbivores and carnivores with a few minor variations.

Neutrophils were the most common circulating immunocyte which reflects the typical mammalian pattern (Coles, 1986). Like dog and porcine neutrophils, dolphin neutrophils were similar in size and irregularly lobed with rare formation of filaments connecting the lobes. A nuclear sex bud was not observed in female dolphins. This structure is often present in dogs and common in some manatees (see Section 4.11). As in other mammalian species band neutrophils were rarely seen in healthy dolphins.

Lymphocytes were the next most common dolphin immunocyte which is a typical mammalian pattern (Coles, 1986). Lymphocytes were composed of a predominant small-type followed by a large-type and a rare granular type. The lymphocyte size and morphology of the three subtypes with Wright-Giemsa stain correlates with the dog and cow

(Coles, 1986). Total lymphocytes were present in relative and absolute numbers similar to terrestrial mammalian species (Jain, 1986).

The dolphin eosinophil was similar in size and morphology to other mammals, however, the mean differential and absolute values were higher, especially in the free-ranging dolphin group. The reason for this is speculative but could be partially explained by reviewing the functions of the eosinophil, keeping in mind that some eosinophil functions remain to be fully defined. Eosinophils are known to have wide phagocyte abilities and bactericidal and parasiticidal effects (Clark and Kaplan, 1975; Roth and Kaeberle, 1981). Eosinophils are also known to be regulators for allergic (i.e., immediate-type hypersensitivity reactions) and inflammatory reactions. Their ability to phagocytize immune complexes probably reduces the availability of antigen for further immune response (Jain, 1986). Eosinophils can produce cell damage through release of "major basic protein" (MBP), during disease states associated with hypereosinophilia (Schatz, 1981). Other less defined eosinophil functions involve a role in fibrinolysis, an inhibitory effect on granulopoiesis and the remodeling of connective tissue in late stages of inflammation (Jain, 1986).

Eosinophilia is common to diseases of tissues and organs containing high concentrations of mast cells, (e.g., the skin, lungs, gastrointestinal tract and female genital organs). Pathologic states associated with interactions between antigen, IgE, and mast cells or basophils are often associated with eosinophilia and include certain metazoan parasitic

infections, allergic respiratory disease, and dermatoses (Olsson and Venge, 1979; Jain, 1986). The cause of the high circulating eosinophils in free-ranging dolphins is, in part, probably due to metazoan parasitism which is common in free-ranging dolphins especially in the pulmonary and gastrointestinal tracts (Dailey and Brownell, 1972). The elevated numbers of circulating eosinophils in parasite-free captive dolphins is a mystery. It is tempting to speculate a role of enhanced pulmonary and gastrointestinal phagocytosis of immune complexes since eosinophils are typically found in these tissues in relatively higher numbers compared with other mammals (Bossart, personal observation). This could reflect an immunologic adaption to the marine aquatic environment where potential and opportunistic pathogens are easily inhaled in aerosol form or swallowed in normal feeding activities.

In the standard leukocyte differential, monocytes were the least numerous leukocyte which reflects a typical mammalian pattern (Coles, 1986). Dolphin monocytes were of similar size and morphology to those of other terrestrial mammals.

Basophils were not identified in any dolphin peripheral blood samples. Basophils are rare peripheral blood immunocytes in domestic animals particularly in the dog and cat (Jain, 1986), but their total absence in this large population of dolphins is unusual in mammals.

In healthy dolphins all lymphocyte subsets (T_h , $T_{c,s}$, and B lymphocytes and NK cells), were identified and had identical polymethine staining characteristics to similar cells in humans and mice.

Circulating T lymphocytes and B lymphocytes comprised 91-97% and 1-6% respectively of total peripheral circulating lymphocytes. These T cell values were similar but somewhat higher than the only other published values, given by Romano, et al. (1992) who concluded that, in healthy dolphins, T lymphocytes comprised 85-90% of total circulating lymphocytes. The B lymphocyte values from this study were less than those reported by Romano, et al. (1992), (1-6% vs. 10-15%, respectively). The lower B lymphocyte value in this study was due partially to the inclusion of NK cells in the lymphocyte subset differential. The NK cells ranged from 1-4% of total circulating lymphocytes. NK cells were not characterized in the Romano study.

Clinically normal dolphin circulating T cells were higher and B cells lower than human, (listed respectively) (60-80% and 5-15%); adult rhesus monkey (Macaca mulatta) (50-78% and 20%); dog (74% and 30%); cat (50% and 30%); pig (55-70% and 9%); buffalo (81% and 5-18%); mouse and bat (Pteropus giganteus) (75-80% and 20-25%) (Jain, 1986; Rappocciolo, et al., 1992; Sariker and Chakravart, 1991).

Clinically normal dolphin peripheral blood T lymphocyte subpopulations were more similar to those of sheep, horse and bovine (83-85% peripheral blood T lymphocytes) (Jain, 1986; Weldon, et al., 1992; Okada, et al., 1993). From an evolutionary standpoint this supports the hypothesis that dolphins have evolved from common ancestors of terrestrial ungulates (Slijper, 1962).

In clinically normal dolphins, the T lymphocyte subsets were comprised of 73-89% T_h cells and 8-21% T_{cy} cells. NK cells were the least numerous lymphocyte comprising 1-

4% of total lymphocytes. This is the first study to document these cells in the peripheral blood of dolphins. Dolphin circulating T_h lymphocyte numbers were higher than the human (42-55%); adult rhesus monkey (25-35%); African green monkey (26-39%) and baboon (32-37%); and sheep (28%) (Rappocciolo, et al., 1992, Okada, et al., 1993).

Circulating dolphin T_{cs} lymphocytes were lower than the adult rhesus monkey (37%), african green monkey (65%) and baboon (74%). Interestingly, dolphin T_{cs} lymphocytes were in the normal range for humans (21%) (Rappocciolo, et al., 1992).

NK cells in clinically normal dolphins were lower than the human (15%), adult rhesus monkey (13%), baboon (23%), and african green monkey (11%) (Rappocciolo, et al., 1992).

The mean $T_h:T_{cs}$ ratio in clinically normal dolphins was 6.5 (range 3.4-10.7). This ratio is higher than reported for humans (>1-2.5%); adult rhesus monkey (0.69-0.94); cat (1.13), adult sheep (1.49); baboon (0.058); and african green monkey (0.35) (Kuhlman, et al., 1991; Tomkins, et al., 1991; Rappocciolo, et al., 1992).

The preponderance of T lymphocytes in clinically normal dolphins compared to terrestrial mammals suggests heightened circulating cell-mediated immunologic functions. Circulating humoral immunity, based on the lower numbers of B lymphocytes is probably not as critical in dolphins and most likely is relegated to lymphoid tissue such as spleen, lymph nodes, and mucosal-associated lymphoid tissue (e.g., Peyers patches). This follows the general mammalian trend in which T lymphocytes represent circulating migratory cells while B lymphocytes are relatively sessile cells located throughout solid lymphoid tissue,

respiratory, and gastrointestinal tracts (Benjamin and Leskowitz, 1988). In this lymphoid tissue, upon antigen stimulation the B lymphocyte proliferates and develops into a plasma cell or a memory cell following a phase of proliferation, activation and blast transformation (Roitt, et al., 1993). Perhaps antigenic challenges to cell-mediated immunity are increased in the marine environment. This might include antigenic challenges from viral and other infectious agents which cause intracellular infection as well as neoplastic disease. These processes typically stimulate cell-mediated immunity versus humoral immunity.

The relatively higher circulating T_h lymphocytes in dolphins suggests the enhanced ability of directly coordinating the cell-mediated immune response and indirectly the humoral immune response. This could function through the effects of T_h cell produced lymphokines on the clonal expansion of T_c cells, B cells and additional T_h cells. Evaluation of circulating lymphokines in dolphins should shed light on this interesting observation. Interleukins which should be evaluated include IL-2, IFN γ , IL-4, IL-5, IL-6, and IL-10. This may shed light on the presence of the T_h clones in dolphins. In mice and man, two groups of T_h cell clones have been found. One secretes IL-2 and IFN γ , the other produces IL-4, IL-5, IL-6, and IL-10 (Romagnini, 1991). These are known as the T_h1 and T_h2 cell subsets, respectively. T_h1 cells mediate several functions associated with cytotoxicity and local inflammatory reactions and are therefore critical for combating viral, bacterial and parasitic infections. T_h2 cells are more effective at stimulating B cells to proliferate and produce antibodies, and therefore function primarily to protect against free-living microorganisms (humoral immunity) (Roitt, et al., 1993; Romagnini, 1991).

The presence of T_{cs} lymphocytes suggests the ability to destroy antigenically-altered cells or cells containing intracellular organisms (through the actions of T cytotoxic cells) and the ability to balance or down regulate the immune system (through the effects produced by T suppressor cell lymphokines). The significance of lower relative circulating numbers of T_{cs} lymphocytes compared with terrestrial mammals is speculative. To add to this speculation, it is currently unclear whether T_s cells really exist as a subpopulation functionally separate from other subsets (Roitt, et al., 1993). As with all of these evaluations, perhaps cell numbers do not reflect overall functional ability of the lymphocytes to perform their designated tasks. Alternatively, non-circulating lymphocyte subpopulations in the dolphin may be able to be mobilized quickly when needed for specific functions. Similar speculations can be made about the comparatively lower numbers of circulating NK cells. Whether NK cell activity is augmented by exposure to neoplastic or virally-infected cells, as in other species, awaits further functional studies (see Section 4.22 and 4.30 for further comments on NK cells).

4.12 MORPHOLOGY AND VALUES OF IMMUNOCYTES IN HEALTHY MANATEES

As with dolphins, the mean total WBC counts were comparable to terrestrial mammals but in the high normal range (7,300-10,905 cells/mm³) (Coles, 1986). However, notable differences were present in the immunocyte morphology with Wright-Giemsa stained specimens and in standard immunocyte differential cell values.

The predominant segmented nucleated cell type was an immunocyte containing small pleomorphic eosinophilic cytoplasmic granules. This cell has been termed a heterophil or heterophilic granulocyte. Recent evidence (Kiehl and Schiller, 1994) demonstrated this cell to stain positive for myeloperoxidase in a manner similar to the human, dog, cat, and horse neutrophil and unlike the rabbit and bird heterophil. Kiehl and Schiller concluded that the predominant segmented cell type of the manatee was not biochemically similar to the heterophil of the bird or rabbit or to the eosinophil of the human, dog, cat, horse, bird and rabbit.

Another recent study also disputes the terminology of heterophil versus neutrophil in the manatee. (Converse, et al., 1994). This study evaluated the Antillean manatee (Trichechus manatus manatus), a subspecies of the Florida manatee of this study. Based on staining characteristics, the authors concluded that this segmented cell with eosinophilic granules more closely resembled a neutrophil. This argument is largely one of semantics since both cells probably have the same functions. Nevertheless, the term heterophilic granulocyte may be a more appropriate morphologic term for this immunocyte in Florida manatees. Also, unlike the Antillean manatees from the above study, a nuclear sex bud (Barr body) was rarely observed in these immunocytes in this study. The significance of this is unknown.

With Wright-Giemsa staining, lymphocytes were the next most numerous immunocyte in the peripheral blood of manatees. The clinically normal manatee had generally higher relative and absolute numbers of circulating total lymphocytes compared

with other mammals (Coles, 1986; Jain, 1986). Interestingly, the elephant, a presumed evolutionary relative of the manatee, has similar total circulating lymphocyte values (44%) and heterophilic granulocytes with demonstratable peroxidase activity (Silva and Kuruwita, 1993a, 1993b).

In healthy manatees all lymphocyte subsets (T_h , T_{cs} , and B lymphocytes and NK cells) were identified and had identical staining characteristics to the similar cells in humans, dolphins and mice. Circulating T cells ranged from 95-96%, B cells from 2-3%, and NK cells 2% of the total lymphocyte population. Of the T lymphocytes, T_h cells were 84-89% and T_{cs} were 7-11% of the total circulating lymphocytes. The relative percentages of lymphocyte subsets were similar to those of dolphins with slightly higher T_h and lower T_{cs} values. However, the absolute lymphocyte subset values in manatees were higher than dolphins due to higher total circulating lymphocyte numbers.

As with dolphins, this is the first study to document these cells in manatees and similar to dolphins, the preponderance of T_h lymphocytes is unusually high and T_{cs} lymphocytes low compared with other terrestrial species which have been studied (see Section 4.10). As with dolphins, circulating manatee NK cells were lower in number than those reported for humans, African green monkey and baboon (Rappocciolo, et al., 1992).

The mean $T_h:T_{cs}$ ratio in clinically normal manatees was 9.9 (range 7.6-12.2) which, as in dolphins, is higher than the $T_h:T_{cs}$ ratio reported for primates (Kuhlman, et al., 1991; Tomkins, et al., 1991; Rappocciolo, et al., 1992). The next most common immunocyte was

the monocyte which had similar morphology and staining characteristics as terrestrial mammals.

The manatee eosinophil was the least numerous circulating immunocyte. This differs from the dolphin where the eosinophil was the third most common circulating immunocyte. It appears the manatee does not have a clearly distinct eosinophil population. In some cases only a subtle difference between acidophils (i.e., heterophilic granulocyte and eosinophilic granulocyte) was present. The significance of this low circulating eosinophil number compared with dolphins and terrestrial mammals is unknown. Manatees are susceptible to various organ helminthic parasitic infections. Perhaps the unique heterophilic granulocyte assumes some of the functional roles of the typical terrestrial mammalian eosinophil in the manatee.

4.20 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS - A REVIEW

A review of the kinetics and functions of mammalian immunocytes is necessary for an interpretation and appreciation of immunocyte dynamics in dolphins and manatees.

Leukocytosis is an increase in total immunocyte count above the normal upper limit for an animal species. Leukocytosis can be physiologic or pathologic. Physiologic leukocytosis can be a result of factors such as age, stress, pregnancy, estrus and stage of digestion (Jain, 1986).

For example, muscular exercise and apprehension influence total and differential immunocyte counts. Under increased physiologic stress, the total immunocyte count may be increased, and the number of neutrophils will be greater than normal as cells move from

the marginal granulocytic pool to the circulating granulocytic pool (Coles, 1986). In some animals (e.g., cow, dog) an increased immunocyte count can also occur during the late stages of pregnancy (Coles, 1986).

Pathologic leukocytosis, as a rule, is an initial increase in mature neutrophils which may be relative -- an increase in percentage -- or absolute -- an increase in the total number of cells or both. Immunocyte population dynamics is generally a reflection of the host's response to disease. In addition to an evaluation of the presence of increased numbers of neutrophils, the stage of maturation (i.e., the presence of immature band neutrophils) must also be taken into consideration, as must any changes in the peripheral population of lymphocytes, eosinophils, and monocytes (see below). The shifts in immunocyte peripheral populations have diagnostic and prognostic significance when coupled with the clinical condition of the patient. Causes of pathologic leukocytosis include generalized or localized infections, intoxications, trauma, rapidly growing neoplasia and leukemias.

As indicated, leukocytosis generally involves an initial relative or absolute increase in neutrophils (neutrophilia). The principle function of the mature neutrophil is phagocytosis as a part of innate or nonspecific immunity. The immature band neutrophil also has some phagocytic ability. Neutrophils also elaborate powerful intracellular and/or extracellular proteolytic enzymes. In systemic infections, neutrophilia is frequently preceded by a decrease in total circulating immunocytes (leukopenia) due to tissue sequestration of immunocytes. Leukopenia is most likely to occur in overwhelming bacterial infections, viral infections or endotoxemia. Peripheral blood neutrophils typically

increase in bacterial infections such as salmonellosis, pasteurellosis and other septicemias (Coles, 1986). An increase in immature band neutrophils may also occur. In localized infections produced by pyogenic bacteria such as staphylococci, the neutrophilia is usually much greater than with systemic diseases (Coles, 1986).

Non-infectious diseases which result in a neutrophilia are usually those which stimulate a "stress" reaction such as metabolic disturbances, drugs, and toxic chemicals. This is generally due to the endogenous release of adrenocorticosteroids which also causes a marked reduction or disappearance of the eosinophils, moderate reduction of lymphocytes, and mild increase in monocytes (Coles, 1986). The neutrophilia response differs markedly between species, for example, the dog may typically have a neutrophilic leukocytosis of 80,000 cells/mm³ (normal =15,000 cells/mm³) (Coles, 1986). On the other hand, the cow is less responsive in terms of total immunocyte count than are other domestic species (Coles, 1986).

"Shift to the left" is a term used to denote an increase in the number of immature neutrophils (band neutrophils) in the peripheral circulation. Two types of left shifts are recognized. A regenerative left shift is characterized by an absolute increase in neutrophils accompanied by the appearance of band neutrophils. A slight left shift indicates a mild to moderate increase in the number of band neutrophils while a marked left shift has increasing numbers of even more immature neutrophils such as metamyelocytes and sometimes myelocytes.

A degenerative left shift is characterized by a normal, low or falling total immunocyte count accompanied by a moderate to marked shift to the left with the absolute band neutrophils frequently exceeding the number of mature neutrophils. This generally reflects the inability of the bone marrow to produce mature cells in response to infection, and often is a poor prognostic indicator.

Lymphocytosis which reflects an increase in the absolute number of circulating total lymphocytes occasionally results in a pathologic leukocytosis in domestic animals. Lymphocytosis sometimes occurs following vaccination and in chronic infections when chronic antigenic stimulation results in an increase in the number of T lymphocytes (Jain, 1986). Other conditions that can cause lymphocytosis include the recovery stages of certain infections, adrenocortical insufficiency, neoplasia (leukemia) and hyperthyroidism (Coles, 1986). The lymphocyte subset cellular dynamics in regard to changes in circulating lymphocyte numbers will be discussed below.

Pathologic leukocytosis can also involve increased circulating numbers of the eosinophils (eosinophilia). Eosinophilia can be seen with hypersensitivity reactions such as parasitism and allergic conditions. Generally, migrating parasites such as ascarid larvae, trichinae, and, occasionally, hookworms will produce an eosinophilia (Tizard, 1986). Parasites that produce only localized lesions do not usually induce an eosinophilia. Allergic conditions such as asthma, urticaria, eosinophilic gastroenteritis, allergic bronchitis, allergic dermatitis and food allergies also produce an eosinophilia (Coles, 1986).

Increases in circulating monocytes (monocytosis) can occur in various chronic diseases. Chronic disease, which produces a large amount of particulate matter, typically causes monocytosis. Some examples include fungal and mycobacterial diseases. Monocytic leukemia, acute stress and hyperadrenocorticoidism can also cause monocytosis. Specific infectious diseases such as erysipelas and listeriosis in swine and brucellosis in cattle can also cause monocytosis (Coles, 1986).

Leukopenia is a reduction in immunocyte count below normal values. Leukopenia either may be balanced (i.e., a decrease in all immunocyte types) or may be confined to a singular cellular element.

In the latter case, this may involve a neutropenia, lymphopenia or eosinopenia. Neutropenia can be a result of ineffective bone marrow production, reduced production, and/or reduced survival in the blood (Jain, 1986). Neutropenia due to reduced survival in the blood can occur in diseases when the demand for neutrophils is such that the bone marrow's storage pool is exhausted and the compensatory functional reaction (i.e., increase in immature neutrophils, followed by a comparative change in neutrophils) has not yet become manifest.

Conditions that may produce leukopenia include viral infections such as canine distemper, infectious canine hepatitis, feline panleukopenia, hog cholera and swine influenza (Coles, 1986). The immunocyte decrease is usually observed early in the disease, followed by a leukocytosis with secondary infections. Overwhelming bacterial infections, gram-

negative bacterial endotoxemias, anaphylactic shock and some chemical agents (e.g., chloramphenicol, phenacetin, cortisone, etc.) can also produce leukopenia (Coles, 1986).

Lymphopenia can be caused by certain viral diseases such as canine distemper, dolphin morbillivirus infection, parvoviral enteritis, and feline leukemia virus infection (Coles, 1986; Jain, 1986). Increased circulating glucocorticoids from stress or exogenous administered sources also results in an absolute decrease in total lymphocytes.

Similarly, eosinopenia occurs in any physiologic or pathologic stress condition that involves elevated circulating glucocorticoids (e.g., emotional "stress", administration of therapeutic cortisone, adrenocortical hyperplasia or functional neoplasia) (Jain, 1986).

Interpretation of peripheral blood immunocyte dynamics produces important data in regards to a disease condition. Essentially, three factors can be elicited in this interpretation including: (1) severity of the disease, (2) duration of the process, and (3) prognosis. The severity of the disease process can be evaluated by total immunocyte numbers and relative cellular proportions. In general, it may be said that the extent of a leukocytosis indicates the degree of an individual's resistance and that the degree of a left shift indicates the severity of an infection. A mild to moderate leukocytosis with a slight left shift with circulating eosinophils suggests a mild infection that is being adequately handled by the body's immune system. A marked leukocytosis with neutrophilia indicates a more severe disease condition with an adequate immunologic response. Alternatively, a marked leukocytosis with mature neutrophilia, lymphopenia, and eosinopenia is consistent with a moderate to severe pathologic condition. A degenerative left shift with or without toxic neutrophils suggests

the disease condition is severe. A most severe disease state scenario is a leukopenia with a marked left shift. Alternatively, normalization of the total immunocyte count with a diminishing proportion of neutrophils and a return of circulating lymphocytes and eosinophils indicates diminishing severity and probable recovery (Coles, 1986; Jain, 1986; Bossart and Dierauf, 1990).

Peripheral blood immunocyte dynamics may shed light on the duration of a disease process. Acute disease conditions may be accompanied by conditions ranging from a balanced leukopenia to a regenerative shift to the left or any situation between. Peracute diseases involving overwhelming bacterial or viral infections or endotoxemias can be accompanied by a marked balanced or unbalanced leukopenia with or without a regenerative left shift. Neutrophilia generally appears if the animal survives and decreased circulating lymphocytes and eosinophils accompanies either physiologic or pathologic "stress".

With most chronic, resolving disease conditions, characteristic alterations include absolute increases in total lymphocytes and monocytes. A chronic, nonresolving disease condition often involves a degenerative left shift and ultimate bone marrow depression. With disease resolution, the number of immature neutrophils decreases and mature neutrophil numbers normalize. As physiologic or pathologic "stress" is reduced, the lymphocyte and eosinophil absolute counts return to normal.

Immunocyte dynamics also have prognostic significance. This prognostic usefulness is generally based on serial peripheral blood immunocyte counts (see Section 4.30). Immunocyte parameters which favor a positive prognosis include falling total immunocytes

with reappearance of lymphocytes and eosinophils, a decrease in immature neutrophils, transitory increase of monocytes and the disappearance of toxic neutrophils (Coles, 1986). Unfavorable prognostic signs include degenerative left shift associated with ineffective granulopoiesis, persistent lymphopenia, persistent circulating toxic neutrophils, persistent eosinopenia, persistent marked leukocytosis with neutrophilia, and persistent leukopenia with decreased numbers of all immunocyte types (Coles, 1986; Jain, 1986).

Determination of peripheral blood lymphocyte subset kinetics is a recent technique that has been found useful in interpretation of immunocyte alterations in various disease states in both humans and some domestic animals. The quantitative and morphologic evaluation of the peripheral blood lymphoid component traditionally has been the clinical measure of immunologic status. However, it must be remembered that the peripheral blood is only one compartment through which lymphocytes circulate. Lymphocytes continuously enter and exit lymphoid and non-lymphoid organs using the blood as a traffic route (Yednock and Rosen, 1989; Pabst and Binns, 1989). Unfortunately, in a clinical situation blood is often the only easily accessible source of lymphocytes.

Lymphocyte kinetics differ greatly from granulocyte kinetics (i.e., neutrophils, eosinophils, basophils) in that lymphocytes are produced in many organs while granulocytes originate from the bone marrow. Lymphocytes have a very short mean transit time through blood (30 minutes) versus granulocytes which can remain in the blood for up to 20 hours (Westermann and Pabst, 1990). Lymphopenia may mean that lymphocytes are accumulating in a non-blood tissue compartment elsewhere. On the other hand, lymphocytosis may mean

that cells are failing to circulate to other compartments properly. In either case, it is best to consider lymphocyte distribution as a dynamic system with blood lymphocyte counts representing a single representative compartment in this complex system. Lymphocyte subset compartment maldistribution can have functional consequences and may be relevant to the pathogenesis of altered immunologic states in certain apparently unrelated diseases. For example, changes in blood lymphocyte subset phenotypes have been shown to be diagnostically useful in humans with lepromatous leprosy, Hodgkin's disease, Crohn's disease, mycosis fungoides, systemic lupus erythematosus, chronic liver disease and AIDS (de Sousa, 1983).

For example, in Hodgkin's disease, the total circulating T lymphocytes decline with a concurrent decrease in the $T_h:T_{cs}$ lymphocyte ratio (i.e., the blood T_h lymphocytes decline while the blood T_{cs} lymphocytes increase). Interestingly, the dynamic nature of this process is demonstrated by an increase in the T_h lymphocytes and decrease in the T_{cs} lymphocytes in the spleen of these patients. The splenic lymphocytes in patients/s with Hodgkin's disease are responsive to mitogen stimulation, but the peripheral blood lymphocytes of the same patients/s are not (de Sousa, 1983). Therefore, the known anomalies of lymphocyte number and function in peripheral blood appear to reflect a maldistribution of T lymphocytes in the body rather than an absolute T lymphocyte deficit (de Sousa, 1983).

Decreases in peripheral blood T lymphocytes also occurs in Crohn's disease, chronic-active hepatitis, rheumatoid arthritis, multiple sclerosis, and tuberculosis. The redistribution of lymphocytes in these diseases occurs in other tissue compartments. For example, in

Crohn's disease, T lymphocytes increase in the gut (i.e., the Crohn's disease target organ); in chronic-active hepatitis, T cells increase in the liver; in rheumatoid arthritis, T cells increase in synovial fluid; in multiple sclerosis, T cells increase in the cerebral spinal fluid; and in tuberculosis, T cells increase in the lungs (de Sousa, 1983). Probably the most well-known human disease which causes a marked change in the peripheral blood $T_h:T_{c,s}$ ratio is AIDS. A decrease in the circulating number of T_h lymphocytes is one of the earliest clinical events in the HIV infection, and this parameter can be used to predict when clinical AIDS will occur (Eyster, et al., 1987; Polk, et al., 1987).

Lymphocyte subset population dynamics have also been described in domestic animal diseases. In domestic cats and African lions (*Panthera leo*) with feline immunodeficiency virus (FIV) infection inverted $T_h:T_{c,s}$ lymphocytes ratios have been reported (Ackley, et al., 1990; Kennedy-Stoskopf, et al., 1994). The average $T_h:T_{c,s}$ lymphocyte ratio in the domestic cat is 1.74 (Tompkins, et al. 1990). In the domestic cat and lion the lymphocyte inversion is due to an increase in $T_{c,s}$ lymphocytes which is characteristic in the long asymptomatic period of FIV infection (Novotney, et al., 1990; Ackley, et al., 1990).

Phenotypic subtyping of lymphocytes has also been described from blood and bronchoalveolar lavage fluid in dogs, sheep and humans (Ozaki, et al., 1992; Guzman, et al., 1992; Lujan, et al., 1993; Vail, et al., 1995). In human beings, many inflammatory lung conditions are associated with increasing $T_h:T_{c,s}$ ratios, whereas some immunosuppressive

syndromes result in a significant decrease in this ratio (Walker, et al., 1992; Ozaki, et al., 1992; Guzman, et al., 1992).

4.21 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS - DOLPHINS

Peripheral blood immunocyte dynamics in acutely ill dolphins with probable or confirmed bacterial infections (H21 group) closely followed a typical mammalian pattern as described in Section 4.20 with some notable exceptions. A pathologic leukocytosis occurred which ranged from 2-3 times above the normal total WBC mean counts for the designated living environment categories (Table 3.512). As in most mammalian species, this pathologic leukocytosis involved a significant absolute increase in mature neutrophils when compared to the healthy dolphin group (H1) in all environmental categories. Interestingly, no "shift to the left" (i.e., presence of immature band neutrophils) was present in this group. This suggests a possible overcompensating ability of the dolphin bone marrow to produce mature neutrophils in response to infection. Alternatively, the suspected bacterial infections may not have been severe enough to result in an increased demand for neutrophils and produce a "shift to the left". Whether these cells were functionally competent to carry out phagocytosis and other neutrophil functions awaits further studies.

Another component of this pathologic leukocytosis was a significant eosinophilia in all environmental categories. This trend is unusual for in most instances of pathologic stress involving bacterial infections an eosinopenia occurs as a result of elevated circulating catecholamines and glucocorticoids (see Section 4.20). Eosinophilia is usually seen with hypersensitivity reactions, allergic conditions, and parasitism. Endoparasitism of various

organ systems is common in free-ranging (Fs and Ff categories) dolphins (Howard, et al., 1983) and could possibly explain a concurrent eosinophilia in these categories if parasitic pathologic lesions were produced. However, parasitism is rarely present in captive dolphins due to disruption of parasite life cycles and antiparasitic medical treatment. Therefore, the concurrent eosinophilia in the captive categories was of an unknown etiology. It is tempting to speculate that in all living categories the eosinophilia resulted from an expanded role of the dolphin eosinophil in addition to the classic roles in allergic and hypersensitivity reactions. Perhaps the dolphin eosinophil has a more active involvement in the inflammatory process, specifically bacterial phagocytosis. Alternatively, since many of the infections were pulmonary, perhaps the eosinophilia is a reflection of the high numbers of resident eosinophil populations observed in the lungs which are released into the peripheral circulation (Bossart, personal observation). Pulmonary eosinophilic infiltrates with eosinophilia has been reported in other species (Jain, 1986).

A significant absolute lymphocytosis was part of the pathologic leukocytosis in both captive dolphin categories but not in the free-ranging dolphin categories. This suggests a more responsive cell-mediated immune system in the captive categories as absolute lymphocytosis does not usually occur until a later time period in most infectious bacterial diseases in other mammals (see Section 4.20). Likewise, an absolute monocytosis was also a component of the pathologic leukocytosis in both captive dolphin categories and in the free-ranging stranded category. This suggests a highly responsive antigen-presenting cell component of the immune system as monocytosis also does not usually occur until late in

a chronic resolving disease state that produces abundant particulate matter (see Section 4.20).

The immunocyte dynamics of lymphocyte subsets in the H21 dolphin group compared to the healthy group revealed subset shifts in each environmental category with no apparent trends. T-helper lymphocytes, $T_{c,s}$ lymphocytes and B lymphocytes significantly increased in Fs, Cb, and Ff categories respectively. Therefore, the absolute lymphocytosis observed in the environmental categories was due to increased numbers of different lymphocyte subsets. This may reflect varying functional demands for different lymphocyte types. For example, in Fs dolphins the significant elevation of T_h cells may have reflected an increased need for coordination of the cell-mediated immune response through cytokine production. Alternatively, the significant elevation of $T_{c,s}$ cells in the Cb dolphin group may have reflected an increased need to destroy antigenically-altered cells or cells containing intracellular organisms and/or a need for down regulating the immune system once the infectious condition was under control. The significant elevation of B lymphocytes in the Ff dolphin group may have reflected an increased demand on the humoral immune system to produce antibodies which could then neutralize extra-cellular organisms (i.e., the suspected bacterial pathogens).

It is possible that a combination of these scenarios were present, taking into consideration the short mean transit time lymphocytes have in the blood and the complex dynamic system of lymphocyte distribution and multicompartmentalization. The $T_h:T_{c,s}$ ratios logically demonstrated similar trends. In the Cc group this ratio remained the same

between H1 and H21 groups. Interestingly, this ratio dropped dramatically in captive born dolphins with probable bacterial infections due to significantly high numbers of T_{cs} cells. Alternatively, the ratio elevated in both free ranging dolphin categories due to relative elevations of T_h cells. The significantly higher numbers of T_h lymphocytes and resultant high $T_h:T_{cs}$ ratio in the nonstranded free ranging category (Ff) suggests that these dolphins may have an increased demand for immunologic responsiveness. This supposition is based on the fact that T_h lymphocytes initiate an immune response provided that antigen is presented in an appropriate fashion and that antigen-sensitive cells such as B lymphocytes or effector T cells are unable to respond to antigen unless also stimulated by a T_h cell (Tizard, 1992).

In conclusion, all H21 dolphins demonstrated the pathologic leukocytosis of acute disease with absolute neutrophilia and eosinophilia without a left shift. These dolphins demonstrated an apparently adequate immunologic response to a likely bacterial infection. In many cases, when appropriate antibiotic therapy was instituted in the captive or stranded categories, the dolphins survived the illness and recovered. Lymphocyte subset kinetics exhibited dramatic shifts, but with no specific common trends between living environment categories. This pattern probably represented a favorable prognostic indicator and again reflected the dynamic state of lymphocyte kinetics in dolphins.

Peripheral blood immunocyte dynamics from acutely ill dolphins with terminal septicemia and/or viral infections (H22 group) followed a typical mammalian pattern with some notable exceptions. All dolphins in this category had a pathologic leukopenia which

generally involved all immunocyte types (i.e., neutropenia, lymphopenia, eosinopenia) without a left shift. As in other mammals, these immunocyte dynamics typically reflect overwhelming viral infection and/or gram-negative bacterial endotoxemias. Dolphin morbillivirus (DMV) infection was diagnosed in 4 of the 5 dolphins in the Cc group with pathologic leukopenia, and bacterial endotoxemia was diagnosed in the remaining dolphin in the Cc group and the 2 dolphins in the Fs group. Interestingly, canine distemper virus which is another species-specific morbillivirus also causes a similar pathologic leukopenia in dogs (Coles, 1986).

Another interesting parameter of immunocyte dynamics in this health category was the markedly depressed $T_h:T_{c,s}$ ratios when compared with the healthy or acutely ill leukocytosis group. This was due to a relative combined depression of T_h cells and elevation of $T_{c,s}$ cells. This might have represented either an unsuccessful attempt of eliminating a viral infection and/or an overwhelming endotoxemia with exhaustion of T_h cells. The relative increase in $T_{c,s}$ cells in the viral cases may have reflected a demand for the destruction of cells containing intracellular organisms (i.e., viruses). Immunologic attack of virus-infected cells by cytotoxic T cells is a well-known function of this lymphocyte subset (Chen, 1990a). Natural killer cells also have antiviral activity which is spontaneous cell-mediated cytotoxicity and serves to provide protection before the development of specific cell-mediated cytotoxicity (Tizard, 1992). However, in this group, NK cell values did not change significantly.

As with the H21 group, immunocyte dynamics in the H22 group shed light on the severity of the disease and have prognostic significance. All of the dolphins in this category died within 48 hours of blood sampling in spite of often intensive medical care. Additionally, the potential effects of administered drugs (e.g., antibiotics, etc.) on immunocyte dynamics can not be overlooked in these cases. In other mammals studied, pathologic leukopenia with decreased numbers of all immunocyte types represents a severe disease state with a poor prognosis (see Section 4.20). This probably also explains the low number of dolphins represented in this health group.

Peripheral blood immunocyte dynamics in chronically ill dolphins (H3) had significant changes in only two cell types compared to the clinically normal dolphin group. These changes were present only in the captive dolphin groups. While the total mean WBC counts were significantly higher in the Cb and Cc groups compared to the healthy group, the Cb group had significantly higher neutrophils, and the Cc group had significantly higher eosinophils. Increased numbers of neutrophils suggested the demand for nonspecific immune system functions, specifically, the production of myeloperoxidase, lysosomal enzymes, and defensins which are bacteriocidal and typically used in phagocytosis. (Tizard, 1992).

Increased numbers of eosinophils suggested a hypersensitivity or allergic reaction including parasitism. These etiologies are unlikely in this disease category which generally involved either chronic ongoing bacterial or fungal infections or resolving bacterial infections. These dolphins also were known to be free of parasites. Therefore, this trend

most likely reflects the previously postulated expanded role of the dolphin eosinophil in the inflammatory process, specifically bacterial phagocytosis.

No other significant trends were present in immunocyte dynamics (including lymphocyte subsets) in the H3 group compared to the healthy group.

Compared with acutely ill dolphins (H21) the total mean WBC counts in the H3 group were significantly lower in both Cb and Cc categories. This trend indicates a probable diminished demand for immunocytes which would be expected for a chronic or resolving inflammatory or infectious disease condition. However, the lower total mean WBC counts in each group were due to different immunocyte cellular dynamics. In the Cb group, the total lymphocytes and monocytes were lower in number, while in the Cc group only the neutrophils were lower in number. This trend suggests that in the Cb group the immunocytes of the cell-mediated and humoral immune systems were in less demand, at least in the peripheral blood compartment. Alternatively, in the Cc group, the significantly lower neutrophils implies that non-specific or innate immune functions such as phagocytosis were not in demand as much as during the immunologic response in a chronic disease. This would be expected as the neutrophilic response is typically seen early in an acute inflammatory or infectious condition (see Section 4.20). Additionally, these cellular differences might reflect prior exposure to similar antigens and an anamnestic response.

The $T_H:T_{cs}$ ratios were approximately the same for chronically ill dolphins compared with the clinically normal group. This suggested a steady state system in the lymphocyte subset dynamics in these health categories. This observation might be anticipated with a

chronic resolving disease condition in which the various demands for lymphocyte subset types has stabilized in the peripheral blood lymphoid compartment.

As with the previous health categories, the immunocyte dynamics of the H3 disease group have prognostic significance. When a lowering of the total WBC count and concurrent normalization of the neutrophils and eosinophils occurred in chronic clinical illness, the prognosis was generally favorable. This observation was supported by the fact that most of the dolphins in that category recovered and ultimately converted to a clinically normal health status. This general trend also follows the immunocyte dynamics described in terrestrial mammals (see Section 4.20).

The immunocyte dynamics of dolphins with potentially altered physiologic states (H4 group) were not significantly different compared to the clinically normal dolphin group. That group consisted of clinically normal male and female dolphins experiencing, at the time of blood collection, potential physiologic "stressors," typically either pregnancy or out-of-habitat transport situations (i.e., short or long distance transportation in specially designed padded, water-filled containers). However, when evaluated by sex, the H4 females had significantly higher mean WBC counts and neutrophils compared to the H4 males. This was consistent with a physiologic "stress" neutrophilic leukocytosis seen occasionally in terrestrial mammals under various conditions including pregnancy (see Section 4.20). Since 18 of the 32 dolphins in this category were pregnant, this could account for the different immunocyte dynamics between these categories.

4.22 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS IN HEALTHY AND DISEASE STATES - MANATEES

Peripheral blood immunocyte dynamics in acutely ill, free-ranging injured manatees (Fs group) with probable or confirmed bacterial infectious (H21 group) closely paralleled the typical mammalian pattern as described in Section 4.20. A significant pathologic leukocytosis occurred in this group which was approximately 2 times above the total WBC mean counts of the healthy group. The relative degree of this leukocytosis was generally less than the H21 dolphin group (Table 3.532). The manatee leukocytosis involved a significant absolute increase in the numbers of heterophilic granulocytes, total lymphocytes, T_h lymphocytes, T_{c1} lymphocytes, NK cells and monocytes. As in dolphins, this represents a broadly reactive immunologic response.

Assuming the morphologically distinct manatee heterophilic granulocyte functions in a similar manner as the typical mammalian neutrophil, the observed increased numbers of these cells would be expected in a bacterial infection. Like neutrophils, these cells would represent the first immunocytes involved in so-called natural (i.e., innate, non-lymphoid) immunity due to their phagocytic abilities. Interestingly, no "shift to the left" was present in this group which supports a similar speculation of a possible bone marrow compensating ability that was made for dolphins.

Unlike dolphins, however, manatees did not mount an eosinophilia in the acutely-ill group. In fact, eosinophils made up a negligible percentage of peripheral blood immunocytes in all manatee health categories. This suggests eosinophils may not play as an important role

in IgE-mediated (i.e., hypersensitivity, allergic or parasitic) reactions since 9 out of 10 of the manatees in this health category were in the free-ranging stranded group (Fs group). In this group, endoparasitism was common, especially of the gastrointestinal tract (e.g., nematodiasis, trematodiasis, and cestodiasis). Alternatively, clinical disease which often activates parasitic infections in other mammals may have suppressed a peripheral eosinophilic response due to the pathologic or physiologic release of endogenous adrenal glucocorticoids (see Section 4.20). A third possibility is that eosinophils in manatees remain marginated in blood vessels of various tissues and only circulate in response to tissue demand.

The absolute lymphocytosis in the acutely ill manatees was due to significant increases in both T-lymphocyte and NK subsets but not B cells. This is in marked contrast to acutely ill dolphins which had an absolute lymphocytosis due to increased numbers of only 1 lymphocyte subset per living environment category. This trend suggests a greatly heightened immunologic responsiveness in manatees if one assumes cell numbers are a reflection of cell function and the disease states represented were matched equally enough to allow for comparisons. The immunologic consequences of this cellular trend could include increased coordination and activation of the humoral and/or cell-mediated immunologic response(s) through T_h cytokine production, destruction of antigenically-altered cells or cells containing intracellular organisms and concurrent immunologic down regulation or "fine-tuning" specific immunologic responses. An interesting finding was significantly elevated numbers of NK cells in the acutely ill manatees compared to the

clinically normal group. The absolute numbers of NK cells were markedly higher in this category than any other manatee or dolphin group. This suggests a demand for non-specific or spontaneous cell-mediated cytotoxicity which would be immediately functional prior to the development of specific cell-mediated cytotoxicity.

The significant increase in monocytes also suggests immunologic responsiveness and the need for tissue macrophages. These antigen-presenting cells could then participate in the macrophage-dependent pathway of cell-mediated immunity.

The $T_h:T_{cs}$ ratio was slightly lower in this health category (H21) compared to the clinically normal manatee group. This was due to disproportional increases in T_{cs} lymphocytes and probably reflects one facet of this broadly reactive and dynamic immunologic response. The type of disease process (e.g., viral, bacterial, etc.) would also have biased the response of the T_c lymphocytes.

Only 1 manatee of the total study population was represented in the acutely ill septicemic/viral health group (H22). While statistical evaluation was impossible in this category, the relative percentages of all immunocytes including lymphocyte subsets remained similar to healthy manatees but with an overall drop in the total circulating immunocytes. This is in contrast to the dolphin H22 group which had an inverted $T_h:T_{cs}$ ratio with the accompanying overall drop in the total circulating immunocytes. This may represent a positive prognostic indicator as the manatee in this health group survived while all of the dolphins in the same health group died.

One of the most interesting findings of this study was the noticeable absence of manatees from the chronic disease category (H3). Considering the overall manatee study population size this was unusual and was in marked contrast to the dolphin study population. Perhaps this reflects the manatees' overall increased absolute circulating numbers of total lymphocytes and lymphocyte subsets which, in turn, could result in a constant state of enhanced immunologic responsiveness. This could permit the manatee to eliminate acute infections quickly and more efficiently than dolphins, hence preventing most chronic disease states. This may also explain why manatees can endure devastating traumatic injuries (i.e. human-related injuries from boat collisions, flood gate impacts, etc.) and recover with only supportive veterinary care. Similar injuries in dolphins would generally be immediately fatal or result in death after a brief clinical period even with intensive critical care intervention.

The immunocyte dynamics from manatees with potentially altered physiologic states (H4) group demonstrated some characteristics of a physiologic "stress" leukogram (see Section 4.20). While the total WBC counts were not significantly different from the clinically normal manatees, significant shifts did occur in immunocyte types. Heterophilic granulocytes were elevated and total lymphocytes and T_h cells were depressed which is a common finding from the effects of elevated serum adrenocortical glucocorticoids in physiologic "stress" conditions (see Section 4.20). The manatees in this group were exclusively in the free-ranging stranded injured group (Fs) and often pregnant and/or transported long distances. These singular or combined factors may have resulted in the physiologic conditions necessary to produce the observed immunocyte dynamics.

4.30 PERIPHERAL BLOOD IMMUNOCYTE DYNAMICS - INDIVIDUAL SERIAL CASE STUDIES

4.31 SERIAL CASE STUDIES - DOLPHINS

The first serial case study was from an approximately 6-year-old female delphinid named "Sharky" (see Table 3.551). "Sharky" was clinically normal (H1) during the 4 sampling periods and, therefore, this data is presented for comparative purposes. "Sharky" was sampled at time intervals ranging from 1 week to 4 months. Mild, yet relatively stable variations in total leukocyte numbers occurred at these sampling times. Immunocyte differential cell numbers were also characterized by mild to moderate variations. These variations, however, were always within the clinically normal healthy dolphin ranges for each cell type. This pattern reflected a dynamic, yet immunologic steady state. It suggested the immunologic capability of being broadly reactive and adaptive.

"Bebe" (see Table 3.551) was an approximately 35-year-old captive-born dolphin which developed a dry, non-productive cough (exacerbated by exercise) and sporadic anorexia in October of 1991. This was the same time a confirmed dolphin morbillivirus epizootic was occurring at the same facility. A physical examination revealed bilateral dry pulmonary rales of both lung fields. Nasal sinus exudate was examined cytologically and contained many degenerate neutrophils and suppurative debris admixed with small bacilli. Bacterial cultures of the exudate yielded a pure culture of Pseudomonas aeruginosa. This bacterial infection of most mammals is generally opportunistic in nature, often associated with immunologic compromise and debilitating conditions (Jones and Hunt, 1983). The

working clinical diagnosis at that time was suppurative bronchopneumonia, either primarily viral (i.e., dolphin morbillivirus) in etiology with a secondary Pseudomonas aeruginosa bacterial component or a primary Pseudomonas aeruginosa infection with concurrent immunologic compromise. "Bebe" was placed on oral ciprofloxacin. This antibiotic was bactericidal to the Pseudomonas aeruginosa based on culture and sensitivity testing.

"Bebe's" CBC on Day 1 of clinical signs (10/7/91) indicated a marked leukocytosis approximately three times above the normal range for healthy dolphins (Table 3.511). The cellular components of this leukocytosis included a pronounced mature absolute neutrophilia and eosinophilia. Neutrophilia is the classic early mammalian immunocyte response to infectious extracellular organisms or toxins (i.e., bacteria and/or their toxic products) (see Section 4.20). Again, an eosinophilic component was a part of this early delphinid response to infection in an animal free of parasitism. This finding supports the expanded role of the dolphin eosinophil, perhaps in bacterial phagocytosis and/or elaboration of enzymes which have a bactericidal/bacteriostatic function. This would be in addition to the eosinophil's classic role in hypersensitivity or allergic reactions.

Interesting dynamic shifts in lymphocyte populations also occurred in "Bebe" on Day 1. While the total lymphocytes were within the normal ranges for a healthy dolphin, the lymphocytes subsets were remarkably abnormal. Compared to the healthy dolphin group, a dramatic elevation of B lymphocytes with a concurrent moderate decline of T_h lymphocytes were present at this time. The T_{cs} and NK cells remained within normal dolphin ranges. The markedly elevated numbers of B cells is consistent with a reactive

humoral immune system response and suggests an increased demand for cellular components necessary for immunoglobulin production. This reaction would be anticipated in a primary or secondary systemic bacterial infection as immunoglobulins typically are the primary immunologic defense for neutralizing extracellular organisms or their toxins (see Section 4.20). In "Bebe," this initial B cell response appeared quite reactive and was associated with a moderate polyclonal hypergammaglobulinemia. On Day 10 and 21, the immunocyte dynamics were characterized by a slowly resolving leukocytosis, neutrophilia and eosinophilia.

Alternatively, the total lymphocyte count increased represented by lymphocyte subset increases in T_H cells and $T_{c,s}$ cells. The B lymphocytes remained at a high level similar to Day 1. This serial pattern was consistent with a slowly diminishing need for phagocytic cells (i.e., neutrophils and probably eosinophils) and reflected a successful innate immunologic response. The lymphocytic parameters demonstrated the apparent complexity of coordinating a responsive combined humoral and cell-mediated immunologic response. The increased numbers of T_H cells probably reflected the need for an enhanced coordination of the immune response through the elaboration of various cytokines with their target effects on other T cells and B cells. The increased numbers of $T_{c,s}$ cells may have represented an increased demand to destroy: 1. antigenically-altered cells and/or, 2. cells containing intracellular organisms (e.g., virally-infected cells), and/or 3. a need to down-regulate the immune system once the infection was being brought under control. In this case, the response to a viral infection was probably the primary factor for the $T_{c,s}$ cell elevation. This

case provides a fascinating, previously undocumented insight of immunocyte dynamics in dolphins and highlights the potential diagnostic importance of peripheral blood lymphocyte phenotyping in dolphins. Additionally, this technique may have prognostic usefulness when evaluating immunocyte serial trends. In this case the immunocyte patterns were positive prognostic indicators. "Bebe" survived this infection and recovered completely.

It was never demonstrated serologically that dolphin morbillivirus was the primary pathogen in "Bebe's" case. However, the pathogenesis of a primary typically opportunistic Pseudomonas aeruginosa pneumonia with an apparently intact uncompromised immune system would be perplexing. It is important to remember throughout this study that the presence of circulating immunocytes in any healthy or disease condition did not necessarily imply that those cells were functional.

The next serial case study was "PJ" (see Table 3.551), an approximately one-year-old, male captive-born dolphin which had identical presenting clinical signs as "Bebe". A pure Pseudomonas aeruginosa was also cultured from the nasal sinus of "PJ." This dolphin was placed on oral ciprofloxacin antibiotic therapy for 21 days. Similar to "Bebe," on Day 1 (12/9/91) of clinical illness a marked leukocytosis with an absolute mature neutrophilia and eosinophilia were present. However, unlike "Bebe," a moderate total lymphocytosis was also present composed of moderate increases in T_h and NK cells when compared to the clinically normal dolphin group. B cells and T_{cs} cells were within normal ranges at this time. On Day 11, an identical cellular pattern was present with one notable exception. At this time an almost 19-fold increase in T_{cs} cells occurred from the previously normal level on Day 1.

From Day 31-39 the leukocytosis slowly resolved with the normalization of neutrophils, total lymphocytes, T_h cells, T_{cs} cells, and NK cells. However, the absolute eosinophilia remained.

Based on serologic and histopathologic data the etiologic agent of the pneumonia in this case was recently demonstrated to be consistent with dolphin morbillivirus infection. The *Pseudomonas aeruginosa*, in this case, was probably a secondary opportunistic pathogen. In addition, as a result of the immunosuppressive nature of this virus (see Section 4.4), a secondary mycotic infection occurred at approximately Day 67. Hematologic data was not available from this sampling period.

With a primary viral etiology defined, the serial lymphocyte subset dynamics revealed interesting trends. The increased numbers of T_h cells represented a demand for coordinating the immune response possibly through the generation of cytotoxic T cells. This would represent a classic regulator/effector cellular immunologic response. This process, however, theoretically takes time which may explain the immediate changes observed in NK cells. The initial elevation of NK cells on Day 1 and Day 11 was almost 10 times those of healthy captive-born dolphins (see Table 3.511). NK cells are thought to be involved with nonspecific or spontaneous cell-mediated cytotoxicity and can lyse a variety of target cells including virally-infected cells. This occurs prior to the development of specific cell-mediated cytotoxicity (i.e., mediated by cytotoxic T cells). Thus, this would be an anticipated change in an early viral infection. The elevation of T_{cs} cells at Day 11 would also be consistent with a systemic viral infection as cytotoxic T cells are responsible for being cytotoxic for virally-infected cells. At this time, the absence of a B cell response to

the secondary bacterial infection cannot be explained except as a manifestation of one of the immunosuppressive effects of this viral infection (see Section 4.4). Despite the fact that an obvious, enhanced nonspecific (NK cell component) and cell-mediated responses occurred early in the disease process, "PJ" continued to suffer various opportunistic infections including pulmonary aspergillosis (i.e., an opportunistic fungal infection) over the next 2 years. He finally died from extensive chronic fibrosing pneumonia with histopathologic lesions suggesting systemic immunologic suppression.

The next case involves "Aphrodite," a 17-year-old female dolphin (Cc) (see Table 3.551). This dolphin had sporadic anorexia and was frequently inattentive; however, no specific abnormalities were ever noted on physical examination, blood work-ups (except the CBCs) and/or other clinicopathologic parameters. On Day 1 (1/4/91) the CBC indicated a moderate leukocytosis with mature absolute neutrophilia and eosinophilia. The total lymphocytes, T_h cells, $T_{c,s}$ cells, B cells and monocytes were within normal dolphin ranges. However, NK cells were moderately elevated. Because of the elevated leukocyte count, "Aphrodite" was placed on oral cephalexin (a broad-spectrum antibiotic) for 21 days. On Day 11, a pronounced total lymphocytosis occurred composed of marked increases in T_h cells and mild increases in $T_{c,s}$ and B cells. Approximately 6 months later, the sporadic undefined medical condition continued and, by then, the dolphin converted to a chronic illness status (H3). A mild leukocytosis was present with a concurrent mature absolute neutrophilia and eosinophilia. The lymphocyte cellular parameters were now essentially within normal dolphin ranges. At this time "Aphrodite" was not on medication. This cyclic

pattern of leukocytosis with primarily mature absolute neutrophilia and eosinophilia occurred again 5 months later and responded partially to oral antibiotics (ciprofloxacin). While the primary medical condition of this dolphin remained unknown, some interesting immunocytes dynamics were observed with time. As in "PJ"'s case, an elevation of NK cells occurred with the initial signs of clinical illness which again suggested the immediate need for nonspecific cell-mediated cytotoxicity (Did this represent a response to a viral infection?). This was followed 11 days later by markedly elevated T_h cells with lesser increased numbers of T_c , and B cells. This could have represented the lag phase for the development of specific cell-mediated cytotoxicity. As in the previous cases, innate immunologic mechanisms were apparent early in the disease process as evidenced by marked absolute elevations of neutrophils and eosinophils. This early elevation of eosinophils seems to be a consistent finding with most early acute and sometimes chronic illnesses in dolphins.

The next case study was "Misty," an approximately 15-year-old female dolphin (Cb) (see Table 3.551). "Misty" displayed mild inattentiveness and sporadic anorexia which is a hallmark syndrome early in most dolphin diseases. On Day 1, blood serum chemistry data indicated a moderate elevation of liver enzymes consistent with an acute necrotizing hepatopathy. The CBC at this time reflected a mild leukocytosis with a mature absolute neutrophilia and mild increases in monocytes and eosinophils. While the total lymphocytes were within normal dolphin ranges, the T_c cells were just outside the high normal range for dolphins. The remaining lymphocyte subsets were normal. The dolphin was placed on

various oral antibiotics over a 2-month period with no significant changes in the CBC or blood liver enzymes. At the end of the second month, the total leukocyte count and immunocyte differential counts were almost normal, but the hepatic serum enzymes remained elevated together with additional serum enzymes consistent with hepatobiliary obstruction. This pattern was consistent with the diagnosis of a chronic progressive hepatopathy of undefined etiology. However, an infectious agent was suspected. This dolphin continued to deteriorate over a 2-year period with signs of progressive liver disease. The histopathologic diagnosis at death was a severe chronic-active fibrosing hepatitis. The etiology was never determined, however, the histologic pattern of the liver lesions suggested a hepadnavirus infection (Bossart, et al., 1990). The serial immunocyte changes in this case were not as dynamic as the previously discussed cases. This possibly reflected the inability of "Misty's" immune system to mount an effective immunologic response to neutralize the viral agent. However, the initial rise of $T_{c,s}$ cells may have represented an abortive attempt at neutralizing the suspected viral infection.

The next case was "Papi," an approximately 27-year-old male dolphin (Cc) that developed similar respiratory signs as "PJ" (see Table 3.551). A similar pattern of marked leukocytosis with mature absolute neutrophilia and eosinophilia was present on Day 1. However, unlike "PJ," a mild to moderate total lymphopenia was present with a marked reduction of T_h cells and a disproportionate increase in $T_{c,s}$ cells and B cells. Additionally, $T_{c,s}$ cells markedly elevated further in the progressive course of this disease (see blood taken on 4/23/91, Table 3.551). A primary viral etiology with a secondary bacterial component

was suspected in this case. The pattern of the immunocyte dynamics suggested an acute ongoing viral infection as described in Section 4.20. With supportive antibiotic therapy the pulmonary condition stabilized over a 4-month period. However, permanent obstructive lung disease resulted from the initial infection. Because of this, the dolphin converted to a chronic disease category (H3) and had sporadic bouts of opportunistic bacterial or fungal pulmonary disease. The predisposition to opportunistic pulmonary disease was thought to be due to the extent of initial pulmonary damage (i.e., reduced lung compliance, reduced mucociliary function, etc.) rather than a secondary immunologic deficiency. This speculation was supported by the observed immunocyte dynamics over the course of the disease.

"Delta" was an approximately 7-year-old female dolphin (Cc) that also developed pulmonary signs identical to "PJ" (see Table 3.551). The immunocyte dynamics of "Delta" followed closely those of "PJ" with one notable exception. The time period of the sustained lymphocytosis with concurrent elevations of T_h cells was prolonged in "Delta's" case which suggests this trend may have prognostic significance. Ultimately, "PJ" died and "Delta" still survives today with chronic, yet stable obstructive pulmonary disease.

"Natua" was a 22-year-old male dolphin (Cb) which had a 2-year history of a nonspecific, noninflammatory liver disease characterized by marked elevation of serum hepatic transaminases prior to the initial blood work-up on 9/12/91 (see Table 3.551). The serial blood CBC's indicated normal total leukocyte counts with normal proportions of all

immunocytes except T_H cells, B cells and NK cells. All of these lymphocyte subsets were absent from the three serial blood collections (Days 1, 8 and 15). This was a unique pattern in this study. While occasional single lymphocyte subsets were absent in dolphin peripheral smears, it was highly unusual to have cells absent from 3 different subsets and three consecutive blood samples. The significance of this is unknown. However, in the absence of any other cellular response in this disease process, this suggests an immunologic disturbance in this dolphin. The histopathologic diagnosis at death was a severe vacuolar hepatopathy (non-inflammatory) which was consistent with a metabolic or toxic etiology. It is tempting to speculate that either an endogenous (i.e., metabolic or infectious) or exogenous intoxication caused the hepatic lesions and the unique lymphocyte subset pattern shifts which, in turn, resulted in an immunologic disturbance.

"Tiaka" was an aged, approximately 30-year-old female delphinid (Cc) that exhibited similar mild inattentiveness and sporadic anorexia. On Day 1, blood chemistries indicated mild hepatic disease. A marked leukocytosis was present with a concurrent mature absolute neutrophilia and absolute monocytosis which suggested a chronic-active inflammatory process. The lymphocyte subsets were within normal healthy ranges at this time. "Tiaka" was put on the injectable antibiotic, trimethoprin-sulfadiazole. On Day 5, the total leukocyte count dropped by almost half, represented primarily by neutrophils which suggested resolution of the acute inflammatory phase. However, the total lymphocyte count also dropped below the normal lymphocyte range which suggested a possible immunologic disturbance. On Day 10, a partial relapse occurred characterized by an increase in the total

leukocytes consisting of neutrophils, monocytes and markedly increased numbers of total lymphocytes (represented by T_h cells, T_{c_s} cells and marked numbers of NK cells). This pattern was consistent with a combined innate and cell-mediated positive immunologic response. It also was a positive prognostic indicator at that time as clinicopathologic signs resolved soon thereafter.

The final case was "Schwartz," an approximately 4-year old delphinid (Fs) that developed cutaneous erysipelas. This is a bacterial disease that, if not treated with antibiotics, can be fatal in cetaceans (Bossart and Eimstad, 1988). "Schwartz" was placed on injectable penicillin which is typically the antibiotic of choice for erysipelas (see Table 3.551). "Schwartz's" serial CBC's indicate essentially a moderate leukocytosis with absolute mature neutrophilia, eosinopenia and balanced increase in lymphocyte subsets. On the final CBC (10/24/91), eosinophils returned in normal numbers in the peripheral circulation. This probably represented the effects of decreased pathologic stress (i.e., reduction in circulating adrenal glucocorticoids) (see Section 4.20). Additionally, monocytes increased at that time which was consistent with a chronic resolving inflammatory process. This represented a dynamic positive immunologic response involving varied innate and cell-mediated cellular components. This pattern also reflected a positive prognosis as this animal recovered completely from this potential lethal bacterial disease.

4.32 SERIAL CASE STUDIES - MANATEES

Serial case studies of ill manatees were uncommon in this study due to the relatively rapid ability of manatees to recover from various disease situations. Only 1 manatee was represented in the acutely-ill (H21) serial study group. No manatees were present in the acutely-ill septicemic group (H22) or the chronically-ill (H3) group which was in marked contrast to the dolphin study population (see Sections 4.21 and 4.22). Additionally, only 1 of the manatees in the study suffered from any natural infectious disease process (see below, the "Orion" case). All the other ill manatees had primary traumatic, human-related injuries (i.e., boat impact trauma, flood gate trauma, etc.) often with secondary infections. This was also in marked contrast to the dolphin group in which natural infectious disease was the most common cause of illness.

The first serial case study was "Acacia" (see Table 3.552). "Acacia" was an approximately 7-year-old female manatee (Fs). In 1987 she was rescued after being struck by a boat and suffered sharp and blunt thoracic trauma which resolved in approximately 2 months with conservative medical therapy. At the time of blood sampling, "Acacia" was clinically normal (H1), and therefore this case will be used for comparative purposes. "Acacia" on 4 sampling dates, at 2 to 9 month intervals, demonstrated a stable total leukocyte count with mild variations in all immunocyte types. However, the variations, in most instances, were mild and always within established ranges for healthy manatees. This supports the concept of a dynamic, yet steady, immunologic system as it relates to circulating immunocytes.

"Marjory" was an approximately 9-year-old female manatee in the stranded-injured category (Fs) (see Table 3.552). "Marjory" was rescued from Coot Bay in the Florida Everglades after being hit by a boat. She presented with multiple rib fractures and a related trauma-induced unilateral pneumothorax. She was suffering from exposure and dehydration as a result of an inability to dive due to a positive buoyancy state which was a result of the pneumothorax. She was also anorectic. On Day 1, the CBC indicated a mild leukocytosis compared with the clinically normal manatee group (see Table 4.302). The heterophilic granulocytes (i.e., neutrophils) were within clinically normal manatee ranges. However, a marked total absolute lymphocytosis was present. Of the circulating immunocytes, 63% (9,513 cells/mm³) were lymphocytes! The normal circulating lymphocyte percentage for healthy manatees in this category was 44% (4,290 cells/mm³). This total lymphocyte population was represented by a dramatic increase in the numbers of T_c lymphocytes (4,566 cells/mm³ versus normal range, 52-1264 cells/mm³) and NK cells. The number of NK cells was one of the highest values seen in this study (2,473 cells/mm³ versus normal range, 0-420 cells/mm³). This lymphocyte pattern suggested a demand at this time for nonspecific or spontaneous cell-mediated cytotoxicity (i.e., NK cell functions). In addition, a need for specific cell-mediated cytotoxicity and/or down-regulation of the immune response was suggested by the increased numbers of T_c lymphocytes. It is unknown whether this immunologic response represents a challenge from foreign antigens (i.e., infectious agents) and/or a response to massive soft tissue and osseous damage from the traumatic injuries. On Day 1, "Marjory" was placed on an injectable third generation cephalosporin antibiotic

(ceftriaxone) for 20 days. This drug has broad spectrum gram-negative and gram-positive bactericidal activity. In addition, she was given fluid and nutritional supportive care twice daily.

On Day 2, the total leukocyte count had only slightly increased; however, the immunocyte cellular differential had noticeably shifted in cell populations. At this time an absolute neutrophilia and monocytosis were present indicating a demand for phagocytic activity, possibly bacterial (neutrophilic function) and traumatic tissue-damage related (monocyte-macrophage function). At this time, the total lymphocyte number was within the normal healthy manatee range. However, the lymphocyte subsets had conspicuously changed from Day 1. The T_h cells increased (4611 cells/mm³ versus normal range, 2282 cells/mm³) while the T_{cs} and NK cells markedly decreased to within healthy manatee ranges (301 cells/mm³ and 50 cells/mm³, respectively). The B cells also decreased but were still within the healthy manatee B cell range. This trend suggests an increased demand for coordinating the immunologic response through the multifaceted functions of T_h cells and a diminished need for non-specific and specific cell-mediated cytotoxicity.

On Day 15, the total leukocyte count continued to rise with a similar immunocyte differential trend. B lymphocytes increased slightly suggesting activation of humoral immunologic components. NK cells also increased slightly at this time.

On Day 21, the total leukocyte number declined to approximately the same value as Day 1. Again, the immunocyte population dynamics changed. The neutrophil and monocyte numbers normalized. A mild total lymphocytosis was now present with elevated numbers

of T_h cells (7,016 cells/mm³ versus normal range, 1,472-6,162 cells/mm³ in the healthy manatee group). The $T_{c,s}$ and B cells remained approximately the same while the NK cells dropped to 0. This pattern suggested a continued demand for T_h cell immunologic response coordination with slow resolution of the chronic-active inflammatory condition. The resolution of chronic-active inflammation is reflected in the normalization of the neutrophil and monocyte numbers and, with the absence of immature neutrophils (band cells), is generally a good prognostic indication. Indeed, at this time "Marjory" was clinically improved with complete resolution of the pneumothorax and healing of the multiple rib fractures. Also, she was now eating on her own. "Marjory" continued her recovery and was ultimately placed in the release program after giving birth to a healthy calf.

The next serial case study presented was "Ashaira" (see Table 3.552). "Ashaira" was an approximately 20-year-old manatee (Fs) who was hit by a boat and recovered clinically from her injuries which included blunt hull trauma, multiple rib fractures and a mild, unilateral pneumothorax. At the first CBC (6/24/91) her medical recovery was complete. However, she was placed in an altered physiologic health category (H4) because she was pregnant (the first trimester of a 13-month gestation period). The total leukocyte counts from all four sampling dates were always within the normal ranges for healthy manatees. However, the immunocyte differential population dynamics often differed dramatically from the healthy manatee group. On Day 1 (6/24/91), "Ashaira" had an absolute neutrophilia (i.e., heterophilic granulocytosis) and total lymphopenia with a normal monocyte count. The lymphopenia was essentially balanced (i.e., below normal ranges for T_h , $T_{c,s}$, B and NK cell

numbers). This may have represented a physiologic neutrophilia which is seen typically in the dog and cow in later stages of pregnancy (Coles, 1986). The balanced lymphopenia may have been a response to one or more physiologic "stressors" (i.e., pregnancy, handling, and/or venipuncture sampling, etc.). Stressful conditions in other mammals typically cause a moderate to marked decrease in the relative and absolute numbers of lymphocytes (Coles, 1986). This is typically the result of the secretion of adrenocortical hormones that cause a dissolution of the cells (see Section 4.20).

On Day 7, the neutrophils and total lymphocytes normalized. Monocyte numbers were unchanged. However, the T_h cell numbers continued to decrease while the T_{cs} and NK cell numbers rebounded to high normal counts and the B cell counts increased to above normal levels. These immunocyte population dynamics are difficult to interpret in light of the absence of clinical illness and/or leukocytosis. A stimulation of humoral immunity (i.e., elevating B lymphocyte numbers) and spontaneous as well as specific cell-mediated cytotoxicity is implied by these dynamic immunocyte population shifts.

On Day 31, the lymphocyte subsets continued to exhibit marked population shifts. The total lymphocyte numbers were just below the normal range for healthy manatees. The T_h , T_{cs} , B and NK cell numbers were now in the normal healthy manatee cell ranges. The neutrophil and monocyte numbers remained normal.

On Day 64, the total lymphocytes were in a low normal range with T_h , T_{cs} , and NK cell numbers. However, the B lymphocyte numbers now were in the high normal range. Neutrophils and monocytes remained within normal manatee ranges.

Overall, the immunocyte population dynamics of "Ashaira" are perplexing. Perhaps "Ashaira" was subclinically ill, particularly at Day 7, and the lymphocyte subset population shifts were reflecting that illness. This speculated immunologic response could be interpreted as being successful in overcoming the problem as clinical illness and leukocytosis were never realized. Alternatively, the observed immunocyte population dynamics may have reflected normal immunologic processes which occur in an early manatee pregnancy. This latter speculation raises questions on the concept of maternal immunologic response to the developing fetus. This is a fascinating area of immunology and requires more study in the manatee. As a sidelight, "Ashaira" was released and had a healthy calf in the wild.

The next case study was "Orion," an approximately 12-year-old male (Fs) manatee (see Table 3.552). "Orion" suffered a condition known as "cold-stress." He was exposed to an abrupt freeze from a passing cold front in January of 1991. Manatees are intolerant to cold weather (see Section 1.0). The "cold-stress" syndrome involves a progressive series of signs, initially lethargy, an apparent decreased metabolism, anorexia, and frequently opportunistic disease and sometimes death. At rescue, "Orion" was in the beginning stages of the "cold-stress" syndrome of signs. Initially, he was placed in an acutely-ill health group due to a mild pneumonia and a mycotic skin infection. These clinical conditions resolved, and he was then placed in the altered physiologic health category (H4) due to the cold exposure experience (approximately 30 days previous to rescue).

On Day 1 (2/19/95) the total leukocyte number was in the healthy manatee normal range. Neutrophils and monocyte numbers were also normal. The total lymphocyte numbers

were moderately decreased (1120 cells/mm³ versus normal range, 1740-6486 cells/mm³ in the healthy manatee group). Of the total lymphocytes, T_h cells were markedly decreased in number (896 cells/mm³ versus normal range, 1472-6162 cells/mm³ in the healthy group). The T_cs, B and NK cells were within normal ranges.

On Day 3, the total leukocyte count, neutrophil and monocyte numbers were within normal healthy manatee ranges. However, the total lymphocyte numbers were markedly decreased (648 cells/mm³) with a concurrent decrease in the number of T_h cells (421 cells/mm³). The numbers of T_cs cells (162 cells/mm³) increased but were still within the normal healthy range.

On Day 33, the total leukocyte, neutrophil and monocyte numbers were still normal. The numbers of total lymphocyte T_h cells, and T_cs cells were still below the normal healthy range but had increased since Day 3. B cell and NK cell numbers were still normal.

As seen in the previous cases, while the total lymphocyte count can remain nearly the same on serial blood samples the lymphocyte subsets can shift in quite dramatic patterns. This may shed light on the functional demands placed on the cellular components of the immune system. In this case, the "cold-stress" may have resulted in a suppression of T_h cells which could explain why manatees with this syndrome often are prone to secondary opportunistic infections. A similar mechanism is seen in humans with HIV infection. A decrease in the circulating number of T_h cells is one of the earliest clinical events in AIDS, and this parameter can be utilized to predict when clinical AIDS with secondary opportunistic infections will occur (Eyster, et al., 1987; Polk, et al., 1987). Alternatively,

even though "Orion" was clinically normal on Day 1, and had a normal total leukocyte count, the observed immunocyte population dynamics may have represented a lag recovery phase in lymphocyte subset normalization resulting from the previous clinical illness.

4.40 LYMPHOID TISSUE COMPARTMENT EVALUATION

The inability to cytologically evaluate the lymphoid touch preparations with the polymethine stain was largely a result of specimen preparation rather than staining failure. Lymphocyte subsets could be identified in rare instances in polymethine stained specimens but the preparations were too thick for accurate cellular differential counts and determination of anatomic distributions (i.e., whether cells were from T or B lymphocyte dependent zones). Perhaps less manual pressure of touching the glass slide to the glass slide to the tissue would prevent this problem, however, considering the glass slides were only lightly and briefly touched to the lymphoid tissues in the first place, this may represent one limiting feature of the polymethine stain methodology.

The histopathologic evaluation of dolphin lymphoid tissue revealed several interesting findings. First, marked lymphoid depletion occurred in both B and T lymphocyte dependent areas of the lymph nodes and to a lesser extent the spleens in 73% of the cases (n=15). Ten of these dolphins died from the primary effects of pneumonia and one from confirmed hepadnavirus hepatitis. In these cases, the histologic pattern of the lymphoid depletion in the lymph nodes involved both the lymphoid follicles (i.e., follicular zone, bone-marrow derived lymphoid system, B lymphocyte dependent zone) and the paracortical

regions (i.e., the thymic-dependent lymphoid system, T lymphocyte dependent zone) (Plates 3.621, 3.622 and 3.623).

In the spleens of these dolphins, the histologic pattern of the lymphoid depletion involved both the germinal centers (B lymphocyte dependent zone) and the periarteriolar lymphoid sheaths (T lymphocyte dependent zone). In other species, this histologic pattern is the morphologic expression of combined humoral and cell-mediated immunodeficiency (Jubb, et al., 1985; King, 1986; Bossart and Schwartz, 1990; Bossart, et al., 1991). The severity of the lymphoid depletion in these dolphins suggested a state of severe combined immunodeficiency which was further supported by superimposed secondary opportunistic bacterial and/or mycotic infections involving the lung (Table 3.622).

Lymphoid depletion in the spleen and lymph nodes has been associated with chronic physiologic or pathologic stress, immunosuppression and cachexia (Selye, 1973; Glick, 1983; Bossart, 1984; Leighton, 1986). In humans and other mammals, combined immunodeficient states may be congenital or acquired (Tomar, 1979). Considering the clinical history of this group, these cases probably represented severe acquired combined immunodeficiency states which in humans are often secondary to malnutrition, chronic infection, cancer, renal disease and/or primary viral agents. Increased susceptibility to multiple opportunistic infections in these cases was generally the ultimate cause of death (King, 1986; Robbins, Cotran, and Kumar, 1979).

Considering the diversity and severity of the other concurrent, often chronic lesions in these dolphins, the lymphoid changes probably reflected a secondary condition that was

a consequence of other disease processes. This speculation was further supported by the recent discovery that 9 of the dolphins with pneumonia were infected with the newly described dolphin morbillivirus (Duignan, et al., 1995).

Morbilliviruses constitute a genus of antigenically related RNA viruses within the family of Paramyxoviridae and include measles virus of humans, rinderpest virus of ruminants, and canine distemper virus of dogs and other carnivores. Dolphin, seal and porpoise morbilliviruses are newly characterized within the past 5 years (Visser, 1993; Visser, et al., 1993). The virus is lymphotropic and causes immunopathologic lesions, including splenic and lymph node lymphoid depletion, leading to immunosuppression, which enables the spread of opportunistic and secondary bacterial and mycotic infections particularly of the respiratory system. (McChesney and Oldstone, 1989). A similar immunohistologic pattern was present in these dolphin morbillivirus cases.

The manatee lymphoid tissue histopathologic evaluation revealed a strikingly opposite histopathologic picture (Table 3.623). First, lymphoid depletion was present in only 2 manatees but was mild and limited either to splenic B cell dependent zones or lymph node T cell dependent zones. Six manatees had the opposite histologic picture of mild to moderate lymph node lymphoid hyperplasia involving both B and T lymphocyte dependent zones (Plates 3.624 and 3.625).

This is consistent with a state of humoral and cell-mediated responsiveness and antigenic stimulation (Jubb, et al., 1985). Since these manatees were essentially healthy immediately preceding the fatal traumatic insult the etiopathogenesis of the lymphoid

hyperplasia could not be determined. Since little is known of manatee lymphoid histopathology, perhaps the mild to moderately hyperplastic lymph nodes reflect a normal condition of heightened immunologic responsiveness and not a pathologic lesion.

5.00 CONCLUSIONS

5.10 GENERAL SUMMARY

Interest in the health of marine mammals has increased in the last decade due in part to the attention given to human impact on the marine environment. Recent mass strandings of several marine mammal species have raised questions on the overall condition of marine mammal health and the extent of which pollution, infectious disease, "stress" and captivity influence the health of these animals (Hokama, et al., 1990; Domingo, et al., 1992; Visser, et al., 1993).

The cetaceans and sirenians spend all of their lives in an aquatic environment. They may be of special interest to comparative immunologists because as evolutionary descendants of primitive terrestrial animals that returned to the sea, they possess immune systems that were originally adapted to a terrestrial existence but "re-evolved" to meet the antigenic challenge of infectious agents and parasites of an aquatic environment. However, little is known about cetacean and sirenian immunity or the effects environmental factors have on immune system structure and function (Cavagnolo, 1979; Romano, et al., 1992; Converse, et al., 1994; Erickson, et al., 1995). Chemical and "stress"-induced immunosuppression have been documented in other animal systems including mice (Lundberg, 1992) and humans (James, et al., 1993). Establishing correlations between infectious disease immunopathogenesis, environmental conditions and immunocompetence in cetaceans and sirenians will be possible only when baseline immunologic values have been determined for healthy animals. These baseline values must include both the

characterization of cellular elements and functional capabilities of the immune system. Additionally once these immunologic parameters have been defined they will provide an indication of an animal's overall general health and possibly prognostic information in a disease situation.

This study has provided the first in depth characterization of the cellular components of the immune system in dolphins and manatees. Immunocyte morphology and baseline values of all circulating blood immunocytes have now been established in clinically normal animals under free-ranging, stranded and captive living conditions. This was accomplished through traditional cytochemical techniques and new methodology validated in this study for differentiation of lymphocyte subpopulations. Additionally, immunocyte population dynamics were described in depth in sick animals with various acute and chronic disease conditions. By comparing these normal baseline immunocyte values with various disease condition values, a unique picture emerged of immunologic cellular response patterns in these marine mammals. These cellular patterns introduce for the first time functional implications of immune system activity under various pathologic or physiologic conditions.

Traditional cytochemical techniques demonstrated that immunocyte morphology and the relative cell numbers in healthy dolphins and manatees are similar to terrestrial mammals with some notable exceptions. First, the manatee heterophilic granulocyte (i.e. neutrophil) appears as a morphologically unique cell type that probably functions similarly to the typical mammalian neutrophil. The function of this unique cell type deserves further investigation. The second exception involves the eosinophil. In dolphins, eosinophils make up a large

population of the circulating immunocytes, but eosinophils are rarely observed in manatees. The dolphin eosinophil may have expanded functions based on its high degree of activity in various disease states. On the other hand, perhaps the traditional eosinophilic functions in the manatee are assumed partially by the morphologically similar heterophilic granulocyte. The next notable difference concerns the lymphocyte. Manatees have generally higher circulating total lymphocytes compared to dolphins and other terrestrial mammals. This is similar to the manatee's evolutionary relative, the elephant. Finally, basophils were never seen in this study of dolphins, and manatees. This is unlike most terrestrial mammals that usually have small numbers of circulating basophils. The significance of this is unknown.

New staining methodology validated in this study permitted, for the first time, the evaluation of lymphocyte subset populations in these animals. All lymphocyte subset types were found in healthy dolphins and manatees but in often significantly different proportions. Dolphin T lymphocyte values from this study were similar to the only other published values (Romano, et al., 1992). Compared to dolphins, however, manatees had higher circulating absolute numbers of T lymphocytes made up of an unusually high number of T_h cells. This suggests that the manatee has an enhanced ability to respond immunologically. A more rapidly reactive immune system may explain the low incidence of naturally occurring infectious disease in the manatee study population and the manatee's ability to withstand often devastating traumatic injury and recover with minimal supportive medical care (Plate 5.101).

With few exceptions, immunocyte types and/or absolute numbers were not different between free-ranging, stranded and captive living condition categories in healthy dolphins and manatees. One exception occurred in free-ranging dolphins. Total immunocyte numbers were significantly higher in free-ranging dolphins due primarily to increased eosinophils and total lymphocytes, specifically T_h cells. This may reflect a response to increased antigenic challenge (i.e., parasitic, bacterial, viral, etc.) in the free-ranging environment. Alternatively, captive living conditions with theoretically decreased antigenic challenge due to good preventative husbandry and medical care programs could have resulted in decreased numbers of these specific immunocytes without apparently affecting overall immunologic responsiveness (i.e., captive dolphins like free-ranging dolphins can and do mount successful and faster immunologic responses to pathogenic agents).

With only two exceptions, no significant differences were found in circulating immunocytes of healthy dolphins and manatees based on sex and age. One exception was that male dolphins had significantly higher numbers of circulating monocytes. Male dolphins may need more monocyte/macrophage lineage cells for phagocytosis of large particulate tissue matter. This tissue would be generated from minor traumatic injuries resulting from social interaction with other male dolphin conspecifics. The other exception was that manatees over 10 years of age had significantly higher numbers of B lymphocytes. Perhaps older manatees have an increased demand for humoral immunologic functions (i.e., increased immunoglobulin). These findings deserve further investigation..

The evaluation of immunocyte dynamics in various disease states produced a fascinating insight into innate, humoral and cell-mediated immunity in these marine mammals. Based on the immunocyte standard deviations observed, a wide variation was present in individual animal responses. This is to be expected from a normally distributed population of animals. It also reflects that the overall immunologic response to any given pathogen is dependant on the host's ability to organize and focus a diverse array of immune cells and soluble products against the pathogen. This ability is complex and may vary with the host.

The lymphocyte subset kinetics were particularly intriguing in the serial case studies and emphasize the complex, broadly reactive yet specific and dynamic nature of the immunologic response. This study also highlights the potential diagnostic importance of peripheral blood lymphocyte phenotyping in dolphins and manatees. Based on this data, lymphocyte phenotyping could be developed into an indicator of cellular immunocompetence in both captive and free-ranging marine animals.

Understanding the interactions, events, and dynamics that alter numbers and functions of immunocytes is the first step in being able to identify animals which are likely to develop disease. The results of this study allow this first step to be taken. This knowledge not only increases the immunologic data base for the manatee and dolphin, but will assist in an assessment of free-ranging and captive population health before morbidity and mortality occur. This health assessment might help researchers identify deleterious etiologic environmental agents such as anthropogenic toxins which impact the immune

system. Similar toxicologic evaluations have been made in mice and humans (James, et al., 1993; Lundberg, et al., 1992). In captive animals, this knowledge will be useful for husbandry and preventative medical programs at oceanariums. It also provides adjunct information required for the development of immunologically-based treatment regimens.

The inability to utilize the lymphocyte phenotyping methodology in primary and secondary lymphoid tissues was disappointing and an obvious limitation of the lymphocyte phenotyping technique. Nevertheless, the histopathologic evaluation of lymphoid tissues in this study produced interesting data. Lymphoid depletion has been reported in secondary lymphoid tissue of cetaceans but its etiology has never been determined (Simpson and Gardener, 1972; Bossart, 1984; Bossart, et al., 1991; Geraci, 1989). Simpson and Gardner (1972) postulated that lymphoid depletion in cetaceans was due to lack of potent immunogenic stimulation. In this study, the pronounced lymph node and splenic lymphoid depletion in dolphins with terminal systemic illness probably represented an acquired immunodeficiency state secondary to the illness (i.e., disease was the predisposing factor to lymphoid depletion). As a result of the immunocompromised state, increased susceptibility to multiple opportunistic fungal or bacterial infections was generally the ultimate cause of death. Interestingly, the histologic pattern of the dolphin lymphoid depletion suggested both humoral and cell-mediated immunocompromise which also supports the concept of an acquired secondary condition with resultant increased susceptibility to opportunistic infections.

On the other hand, the manatee lymphoid tissue often revealed a strikingly opposite histopathologic picture (i.e., lymphoid hyperplasia) which suggested a reactive immunologic response of this lymphoid compartment. Whether this represented a sampling artifact in the manatee population or reflected the manatee's ability to mount a successful immunologic defense remains unknown and warrants further studies.

Plate 5.101 - Severe boat-related sharp trauma to fluke musculature and the distal vertebral column in a young manatee. This manatee recovered with supportive care only.

5.20 RESEARCH NEEDS AND RECOMMENDATIONS

This study has demonstrated that a phenotypic examination of the immune system with functional implications can be achieved through evaluation of easily accessible peripheral blood samples and these, in turn, can be used as a gauge of marine mammal health. The investigation of the extent that pollution, infectious disease, “stress” and captivity influences health requires not only a phenotypic examination of the immune system but a determination of the functional capability of immune system.

Assays capable of measuring immune cell function should be developed to establish values for immunocompetence. Proliferation in response to mitogen stimulation has been used to evaluate immune responses in bottlenose dolphins and harbor seals (*Phoca vitulina*) (Colgrove, 1978; Ross, et al., 1993; Lahvis, et al., 1993). Proliferation assays have limitations for deriving functional implications of immunity since they only measure the overall ability of an immune cell population to divide in response to a non-specific stimulus. Since lymphocytes and monocytes in this population do not all have the same proliferative capacity and an assessment of proliferation does not measure other activated immune system components, it is important to develop methods to evaluate responses by particular immunocyte subsets and responses other than proliferation. Therefore other functional tests which should be developed include cytotoxicity, cytokine receptor expression and cytokine production assays. This information can then be combined with phenotypic and proliferation data to yield a more comprehensive picture of the functional potential of a population of immunocytes. Additionally, development of monoclonal antibodies specific

for immunocyte differentiation and activation antigens will complement this study and permit a thorough phenotypic and functional examination of marine mammal immunity.

6.00 LITERATURE CITED

Ackley, C.D., Yamamoto, J.K., Levy, N., Pedersen, N.C., and Cooper, M.D. 1990. Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. *J. Virol.* 64:5652-5.

AFIP Letter. 1994. AFIP scientists discover cause of recent dolphin deaths. 152(4):7.

Asper, E.D., Cornell, L.H., Duffield, D.A., Odell, D.K., Joseph, B.E., Stark, B., and Perry, C.A. 1990. Blood values. In: Leatherwood S. and Reeves R. (eds). *The Bottlenose Dolphin*. Academic Press, San Diego, Ca. pp 479-485.

Beck, C., Bonde, R. and Odell, D. 1981. Manatee mortality in Florida during 1978. In: Brownell, R. and Ralls, K. (eds). *The West Indian Manatee in Florida: Proceedings of a Workshop Held in Orlando, Florida*. Florida Department of Natural Resources. 154 pp.

Beck, C., Bonde, R. and Rathbun, G. 1982. Analysis of propeller wounds on manatees in Florida. *J. Wild. Management*. 46(2):531-535.

Benjamini, E. and Leskowitz, S. 1988. *Immunology - A Short Course*. Alan R. Liss, Inc., New York, N.Y. 390 pp.

- Beurgelt, K., Bonde, R., Beck, C. and O'Shea T. 1984. Pathologic findings in manatees. J. Amer. Vet. Med. Assoc. 185(11):1331-1334.
- Bossart, G. 1982. A treatment regimen for candidiasis in Atlantic bottlenose dolphins. J. Amer. Vet. Med. Assoc. 181:1397-1401.
- Bossart, G. 1984. A suspected acquired immunodeficiency in an Atlantic bottlenose dolphin with lobomycosis and chronic-active hepatitis. J. Amer. Vet. Med. Assoc. 185:1413-1414.
- Bossart, G., Odell, D., and Altman, N. 1985. Cardiomyopathy in stranded pygmy and dwarf sperm whales. J. Amer. Vet. Med. Assoc. 187:1137-1140.
- Bossart, G. and Eimstad, E. 1988. Erysipelothrix vesicular glossitis in a killer whale. Jour. Zoo An. Med. 19(1-2):42-47.
- Bossart, G., Brawner, T., Cabal, C., Caron, J., Trimm, M., and Bradley, P. 1990. Hepatitis B-like infection in a Pacific white-sided dolphin. J. Amer. Vet. Med. Assoc. 196(1):127-130.

Bossart, G. and Dierauf, L. 1990. Marine mammal clinical laboratory medicine. In: Dierauf, L. (ed). Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation. CRC Press. Boca Raton, Fl. pp 1-53.

Bossart, G. and Schwartz, J. 1990. Acute necrotizing enteritis associated with suspected coronavirus infection in three harbor seals. Jour. Zoo Wildl. Med. 21(1):84-87.

Bossart, G., Odell, D., Walsh, M., Lynch, J., Beusse, D., Friday, R. and Young, G. 1991. The histopathologic findings in a mass stranding of pilot whales (Globicephala macrorhynchus). NOAA Technical Report NMFS. 98:85-90.

Burn, D. 1986. The digestive strategy and efficiency of the West Indian manatee. Comp. Biochem. Physiol. 85A(1):139-142.

Burn, D. and Odell, D. 1987. Volatile fatty acid concentrations in the digestive tract of the West Indian manatee. Comp. Biochem. Physiol. 88(1):47-49.

Butcher, E. 1990. Cellular and molecular mechanisms that direct leukocyte traffic. Am. J. Path. 136(1):3-11.

Caldwell, D. and Caldwell, M. 1985. Manatees. In: Ridgway, S. and Harrison R. (eds). Handbook of Marine Mammals. Vol. 3. the Sirenians and Baleen Whales. Academic Press, Orlando, Fl. pp 33-66.

Cardeñac, P., White, J., Francis-Floyd, R. 1984. Initial information on the reproductive biology of the Florida manatee. IAAAM Proceedings. 1(1):35-42.

Cavagnolo, R.Z. 1979. The immunology of marine mammals. Dev. Comp. Immunol. 3:245-257.

Chen, Suio-Ling. 1990a. Cells and tissues of the immune system. In: Sheehan, C. (ed). Clinical Immunology. J.B. Lippincott Co., Philadelphia, Pa. pp 19-29.

Chen, Suio-Ling. 1990b. Mechanisms of the specific immune response. In: Sheehan, C. (ed). Clinical Immunology. J.B. Lippincott Co., Philadelphia, Pa. pp 30-43.

Clark, R.A.F. and Kaplan, A.P. 1975. Eosinophil leucocytes: structure and function. Clinical Hematol. 4:635.

Coles, E.H. 1986. Veterinary Clinical Pathology. W.R. Saunders Co., Philadelphia, Pa. pp. 43-79.

Colgrove, C.S. 1978. Stimulation of lymphocytes from dolphin (Tursiops truncatus) by phytomitogens. Am. J. Vet. Res. 39: 141-144.

Converse, L.S., Fernandes, P.S., MacWilliams, P.S. and Bossart, G.D. 1994. Hematology, serum chemistry and morphometric reference values for Antillean manatees (Trichechus manatus manatus). Journal of Zoo and Wildlife Medicine. 25(3):423-431.

Daily, M.D. and Brownell, R.L. 1972. A checklist of marine mammal parasites. In: Ridgway, S.H. (Ed). Mammals of the Sea - Biology and Medicine. Charles Thomas, Springfield, Il. pp. 528-589.

de Sousa, M. 1981. Lymphocyte circulation: experimental and clinical aspects. John Wiley and Sons, Inc., Chichester, England. pp 47-63.

de Sousa, M. 1983. Lymphocyte maldistribution and immunodeficiency. In: Dixon, F.S. and Fisher, D.W. (eds). The Biology of Immunologic Disease. H.P. Publishing Co., Inc., New York, N.Y. pp 117-125.

Dinareлло, C.A. 1988. Biology of Interleukin 1. FASEB J. 2:108-115.

Dinareлло, C.A. and Mier, S.W. 1987. Lymphokines. N. Engl. J. Med. 317:940.

Domingo, M., Visa, J., Primarola, M., Marco, A.J., Ferrer, L., Rabanal, R. and Kennedy, S. 1992. Pathologic and immunocytochemical studies of morbillivirus infection in striped dolphins (Stenella coercoleoalla). *Veterinary Pathology*. 29:1-10.

Duignan, P.J., House, C., Geraci, J.R., Early, G., Copland, H.G, Walsh, M.T., Bossart, G.D., Cray, C., Sadove, S., St. Aubin, D.S., and Moore, M. 1995. Morbillivirus infection in two species of pilot whales (Globicephala sp.) from the Western Atlantic. *Marine Mammal Science*, accepted for publication.

Dungworth, D.L. 1993. The respiratory system. In: Jubb, K.V.F., Kennedy, P.C. and Palmer, N. (eds). *Pathology of Domestic Animals*. Vol. 2. Academic Press, San Diego, Ca. pp 617-623.

Erber, W., Pinching, A. and Mason, D. 1984. Immunocytochemical detection of T and B cell populations in routine blood smears. *Lancet*. 1:1042.

Erickson, K.L., Di Molfetto-Landon., Wells, R.S., Reidarson, T., Stott, J.L. and Ferrick, D.A. 1995. Development of an Interleukin-2 receptor expression assay and its use in evaluation of cellular immune responses in bottlenose dolphins (Tursiops truncatus). *Journal of Wildlife Diseases*. 31: 142-149.

Eyster, M., Gail, M., Ballard, J., Al-Mondhiry, H. and Goedert, J. 1987. Natural history of human immunodeficiency virus infections in hemophiliacs: effects of T cell subsets, platelet counts, and age. *Ann. Int. Med.* 107:1.

Farmer, M., Wever, R., Bonaventure, J., Best, R. and Domingo, D. 1979. Functional properties of hemoglobin and whole blood in an aquatic mammal, the Amazonian manatee. *Comp. Biochem. Physiol.* 62A:231-238.

Fawcett, D. 1942a. A comparative study of blood-vascular bundles in the Florida manatee and in certain cetaceans and edentates. *J. Morph.* 71:105-124.

Fawcett, D. 1942b. The amedullary bones of the Florida manatee. *Am. J. Anat.* 71:271-309.

Fenwick, B., Russell, S., Johnson, H., Beurgelt, C. and Pontzer, C. 1986. Macrophage mediated immunologic defense mechanisms in dolphins. Research Proposal Submitted to the Office of Naval Research.

Fike, D.J. 1990. Cellular assays. In: Sheehan, C. (ed). *Clinical Immunology*. J.B. Lippincott Co., Philadelphia, Pa. pp. 52-75.

Flynn, S. 1990. Hematolymphoid antigens. In: True, L. (ed) Diagnostic Immunohistopathology. Lippincott Co., Philadelphia, Pa. pp 10.2-10.16.

Gallivan, G., Best, R. and Kanwisher, J. 1983. Temperature regulation in the Amazonian manatee. *Physiol. Zool.* 56(2):255-262.

Gallivan, G. and Best, R. 1981. Metabolism and respiration in the Amazonian manatee. *ACTA Amazonia.* 11(1):103-111.

Gatenby, P.A. 1989. Reduced CD4+ T cells and candidiasis in the absence of HIV infection. *Lancet.* 1027-1028.

Geraci, J.R. and Medway, W. 1974. Simulated field blood studies in the bottlenose dolphin: changes in hematology and chemistry during blood and plasma storage. *J. Wildlife Dis.* 10:410-419.

Geraci, J.R. and Sweeney, J. 1986. Clinical techniques. In: Fowler, M.E. (ed). *Zoo and Wild Animal Medicine.* W. B. Saunders Co., Philadelphia, Pa. pp 771-777.

Geraci, J.R. 1989. Clinical investigation of the 1987-1988 mass mortality of bottlenose dolphins along the U.S. Central and South Atlantic coast. Final report to the National Marine Fisheries Service, U.S. Navy, Office of Naval Research and Marine Mammal Commission.

Gingerich, P.D., Wells, N.A., Russell, D.E. and Ibrahim-Shah, S.M. 1983. Origin of whales in epicontinental remnant seas: new evidence from the early Eocene of Pakistan. *Science*. 220:403-405.

Giorgi, J.V. and Detels, R. 1989. T-cell subset alterations in HIV-infected homosexual men: NIAID multicenter AIDS cohort study. *Clin. Immunol. Immunopathol.* 52:10-18.

Glick, B. 1983. Bursa of Fabricius. In: Farner, D.S., King, J.R. and Parkes, K.C. (eds). *Avian Biology*. Acad. Press, New York, N.Y. pp. 107-119.

Green, D.R., Flood, P.M. and Gershon, R.R. 1983. Immunoregulatory T cell pathways. *Annu. Rev. Immunol.* 1: 439 pp.

Gupta, S., Paul, W. and Fauci, A. 1987. *Mechanisms of Lymphocyte Activation and Immune Regulation*. Plenum Press, New York, N.Y. 417 pp.

Guzman, S., Wang, Y., Teschler, H., Kienast, K., Brockmeyer, N. and Costable, U. 1992. Phenotypic analysis of bronchoalveolar lavage lymphocytes from acquired immunodeficiency patients with and without Pneumocystis carinii pneumonia. Acta. Cytol. 36:900-904 pp.

Hansen, L.J. 1992. Report on the investigation of the 1990 Gulf of Mexico Bottlenose dolphin strandings. NOAA, NMFS, Contribution: MIA - 92/93, 219 pp.

Hartman, D. 1979. Ecology and behavior of the manatee. Special Publication No. 5. Amer. Soc. Mammal. 153 pp.

Herberman, R.B. 1983. Natural killer cells. In: Dixon, F.S. and Fisher, D.W. (eds). The Biology of Immunologic Disease. H.P. Publishing Co., Inc., New York, N.Y. 75-85 pp.

Hohn, D., Cohen, E., Cunningham, R. and Fitzpatrick, J. 1983. Human lymphocyte agglutinins in Tursiops truncatus. Immunol. Comm. 12:481-490 pp.

Hokama, Y., Asahina, A.Y., Hong, T.W., Katsura, K., Shang, E., Miyahara, T., Sweeney, J. and Stone, R. 1990. Causative toxin(s) in the death of two Atlantic dolphins. Journal of Clinical Laboratory Analysis. 4: 474-478 pp.

Horton, J. 1985. Evolution of immunity. In: Roitt, I.M., Brostoff, J. and Male, D. (eds). Immunology. Glower Medical Publishing, London. 91 pp.

Howard, E.B., Britt, J.O. and Matsumoto, G. 1983. Parasitic diseases. In: Pathobiology of Marine Mammal Diseases, Vol. I. CRC Press, Boca Raton, Fl. pp. 119-133.

Husar, S. 1978. Trichechus manatus. Am. Soc. Mammal., Mammalian Species. 93:1-5.

Irvine, A., Odell, D. and Campbell, H. 1981. Manatee mortality in the southeastern United States. In: Brownell, R. and Ralls (eds). The West Indian manatee in Florida: Workshop Proceedings 1978. Florida Department of Natural Resources, Tallahassee, Fl. 154pp.

Irvine, A. 1983. Manatee metabolism and its influence on distribution in Florida. Biol. Conser. 25:315-334.

Jaffe, E. 1985. Surgical Pathology of the Lymph Nodes and Related Organs. Saunders Co., Philadelphia Pa. Pp. 112-122.

Jain, N.C. 1986. Schalm's Veterinary Hematology. Lea and Febiger, Philadelphia Pa. pp. 677-729.

James, R.C., Busch, H., Tamburro, C.H., Roberts, S.M., Schell, J.D. and Harbison, R.D. 1993. Polychlorinated biphenyl exposure and human disease. *Journal of Occupational Medicine*. 35: 136-178.

Jones, T.C. and Hunt, R.D. 1983. *Veterinary Pathology*. Lea and Febinger, Philadelphia, Pa. p. 621.

Jubb, K.V.F., Kennedy, P. and Palmer N. 1985. *Pathology of Domestic Animals*. Vols 2 and 3. Acad. Press, Orlando, Fl. pp. 617-623(2), 223(3).

Kass, L. 1988. Identification of lymphocyte sub-populations with a polymethine dye. *Jour. Histochem. Cytochem.* 36(7):711-715.

Kennedy-Stoskopf, S., Gebhard, D.H. and English, R.V. 1994. Clinical implications of feline immunodeficiency virus infection in African lions (Panthera leo): Preliminary findings. *Proceedings of the American Association of Zoo Veterinarians*. pp. 345-346.

Kiehl, A.R. and Schiller, C.A. 1994. A study of manatee leukocytes using peroxidase stain. *Veterinary Clinical Pathology*. 23(2):50-53.

King, N.W. 1986. Simian models of acquired immunodeficiency syndrome: a review. *Vet. Pathol.* 23:345-353.

Kuhlman, G., Roth, J. and Nissen, S. 1991. Effects of ketoisocaproate on adrenocorticotropism - induced suppression of lymphocyte function in sheep. *Am. Jour. Vet. Res.* 3:338-392.

Lahvis, G.P., Wells, R.S., Casper, D. and Via, C.S. 1993. In vitro lymphocyte response of bottlenose dolphins (Tursiops truncatus): mitogen-induced proliferation. *Marine Environmental Research.* 35: 115-119.

Lazarus, A.H. and Baines, M.G. 1985. Studies on the mechanism of specificity for tumor cells: correlation between target cell transfer in receptor expression and competitive activity. *Cell. Immunol.* 96:255.

Leighton, F.A. 1986. Clinical, gross, and histological findings in herring gulls and Atlantic puffins that ingested Prudhoe Bay crude oil. *Vet. Pathol.* 23:254-263.

Lipscomb, T.S. 1994. Personal communication.

Lujan, L., Begora, I., Collie, D.D.S. and Watt, N. J. 1993. Phenotypic analysis of cells of bronchoalveolar lavage fluid and peripheral blood of maedi visna-infected sheep. Clin. Exp. Immunol. 91:272-276.

Lundberg, K., Deneker, L. and Gronvik, K. 1992. 2, 3, 7, 8 Tetrachlofodibenzo-p-dioxin (TCDD) inhibits the activation of antigen specific T cells in mice. International Journal of Immunopharmacology. 14:699-705.

MacNeill, A. 1975. Blood values for some captive cetaceans. Can. Vet. J. 16:187.

Marine Mammal Commission-Annual Report to Congress. 1995. Marine Mammal Commission, 1825 Connecticut Ave., N.W., Washington, D.C. pp. 17-26.

McChesney, B. and Oldstone, M.B.A. 1989. Virus induced immunosuppression: infections with measles virus and human immunodeficiency virus. Adv. Imm. 45:335-380.

Medway, W. and Geraci, J. 1964. Hematology of the bottlenose dolphin. Am. J. Physiol. 207:1367.

Medway, W. and Geraci, J. 1965. Blood chemistry of the bottlenose dolphin. Am. J. Physiol. 209:169.

Medway, W. and Geraci, J. 1966. Hematology of the bottlenose dolphin. *Vet. Med. Small Anim. Clinic.* 61:557-561.

Medway, W. and Black, D. 1982. Hematology of the West Indian manatee. *Vet. Clin. Path.* 11:11-15.

Medway, W., Dodds, W., Moynihan, A. and Bonde, R. 1982. Blood coagulation of the West Indian manatee. *Cornell Vet.* 72:120-127.

Medway, W., Bruss, M.L., Bengtson, J. and Black, D. 1982. Blood chemistry of the West Indian manatee. *Jour. Wildl. Dis.* 18(2):229-223.

Medway, W. and Geraci, J. 1986. Clinical pathology. In: Fowler, M.E. (ed). *Zoo and Wild Animal Medicine*. W.B. Saunders Co., Philadelphia, Pa. pp. 791-797.

Medway, W., Bossart, G., Hill, E., Hofman, R., Lee, L., Reif, J. and Wells, R. 1990. Independent Review Committee-Findings and Recommendations on the Investigation of Bottlenose Dolphin Deaths in the Gulf of Mexico. Report to the National Marine Fisheries Service, NOAA and the U.S. Marine Mammal Commission.

Morimoto, C., Hafler, D.A., Weiner, H.H., Letvin, N.L., Hagan, M., Daley, J. and Schlossman, S.F. 1987. Selective loss of the suppressor-inducer T-cell subset in progressive multiple sclerosis. *New Engl. J. Med.* 316:67-72.

Mumford, D.M., Stockman, G.D., Barsales, P.B., Whitman, T. and Wilbur, J.R. 1975. Lymphocyte transformation studies of sea mammal blood. *Experimental.* 31:498.

National Marine Fisheries Service. 1990. Final Environmental Impact Statement on the Use of Marine Mammals in Swim-with-the-Dolphin Programs. 1990. pp. 10-69.

Novotney, C., English, R., Housman, J., Davidson, M.G., Nasisse, M.P., Jenq, C.R., Davis, W.C. and Thompkins, M.B. 1990. Lymphocyte population changes in cats naturally infected with feline immunodeficiency virus. *AIDS.* 4:1213-1218.

Okada, K., Ikeyama, S., Ohishi, K., Suzuki, H., Sugimoto, M., Numakunai, S., Chiba, T., Davis, W.C. and Ohshima, K. 1993. Involvement of CD8 T cells in delayed-type hypersensitivity responses against bovine leukemia virus. *Vet.Path.* 30:104-110.

Olsson, I. and Venge, P. 1979. The role of the eosinophil in the inflammatory reaction. *Allergy.* 34:353.

O'Shea, T., Beck, C., Bonde, R., Kochman, H. and Odell, D. 1985. An analysis of manatee mortality patterns in Florida. 1976-1981. *J. Wild. Manage* 49(1):1-11.

Ozaki, T., Tani, K., Nakahira, S., Oqushi, F., Yasuoka, S. and Ogura, T. 1992. Differential cell analysis in bronchoalveolar lavage fluid from pulmonary lesions of patients with tuberculosis. *Chest*. 102:54-59.

Pabst, R. and Binns, R.M. 1989. Heterogeneity of lymphocyte homing physiology: Several mechanisms operate in the control of migration to lymphoid and non-lymphoid organs in vivo. *Immunol Rev.* 108: 83-109.

Piggins, D., Muntz, W. and Best, R. 1983. Physical and morphological aspects of the eye of the manatee. *Mar. Behav. Physiol.* 9:111-130.

Polk, B., Fox, R., Brookmeyer, R., Kanchanaraksa, S., Kaslow, R., Visscher, B., Rinaldo, C. and Phair, J. 1987. Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men. *N. Eng. J. Med.* 316:61.

Quiring, C. and Harlan, B. 1953. On the anatomy of the manatee. *Jour. Mammol.* 34(1):192-203.

Rappocciolo, G., Allan, J.S. and Eichberg, J.W. 1992. A comparative study of natural killer cell activity, lymphoproliferation, and cell phenotypes in nonhuman primates. *Vet. Pathol.* 29:53-59.

Reinherz, E.L. and Schlossman, S. 1981. The differentiation and function of human T lymphocytes. *Cell.* 19:821.

Robbins, W.L., Cotran, R.S. and Kumar, V. 1979. *Pathologic Basis of Disease*. W.B. Saunders, Philadelphia, Pa. pp. 703-753.

Roitt, I.M. 1988. *Essential Immunology*. Blackwell Scientific Publications. London. 286 pp..

Roitt, I.M., Brostoff, J., and Male, D.K. 1993. *Immunology*. Mosby. Philadelphia, Pa. pp. 2.4-2.7.

Romagnini, S. 1991. Human T_H1 and T_H2 subsets: doubt no more. *Immunology Today*. 11: 256.

Romano, T.A., Ridgway, S.H. and Quaranta, V. 1992. MHC Class II molecules and immunoglobulins on peripheral blood lymphocytes of the bottlenosed dolphin (Tursiops truncatus). *Jour. Exper. Zool.* 263:96-104.

Ronald, K., Selly, L. and Amoroso, E. 1978. Biological synopsis of the manatee. Inter. Devel. Res. Centre, Cambridge. pp. 22-25.

Ross, P.S., Pohajdak, B., Bowen, W.D. and Addison, R.F. 1993. Immune function in free-ranging harbor seals (Phoca vitulina). Journal of Wildlife Disease. 29:21-29.

Roth, J.A. and Kaeberle, M.L. 1981. Isolation of neutrophils and eosinophils from the peripheral blood of cattle and comparison of functional activities. J.Immunol.Methods. 45:153.

Sariker, S.K. and Chakravartz, A.K. 1991. Analysis of immunocompetent cells in the bat (pteropus giganteus). Dev. and Comp. Immunology. 15:423-430.

Schatz, M. 1981. Eosinophils and immunology lung disease. Med.Clin.North Amer. 65:1055.

Schloander, P. and Irving, L. 1941. Experimental investigations on the respiration and diving of the Florida manatee. J. Cell. Comp. Physiol. 17(2):169-191.

Selye, H. 1973. The evolution of the stress concept. Am. Sci. 61:692.

Silva, I.D. and Kuruwita V.Y. 1993a. Hematology, plasma and serum biochemistry values in free-ranging elephants (Elephas maximus ceylonicus) in Sri Lanka. Journal of Zoo and Wildlife Medicine. 24(4):434-439.

Silva, I.D. and Kuruwita V.Y. 1993b. Hematology, plasma and serum biochemistry values in domesticated elephants (Elephas maximus ceylonicus) in Sri Lanka. Journal of Zoo and Wildlife Medicine. 24(4) 440-444.

Simpson, J.G., and Gardner, M.B. 1972. Comparative anatomy of selected marine mammals. In: Ridgway, S.H. (ed.). Mammals of the Sea: Biology and Medicine. Springfield, Il. pp. 207-269.

Slijper, E.J. 1962. Evolution and external appearance. In: Whales. Basic Books, New York, N.Y. pp. 58-92.

Sun, T., Li, C., Yam, L. 1985. Atlas of Cytochemistry and Immunocytochemistry of Hematologic Neoplasms. ASCP Press, Chicago, Il. pp. 462-473

Sweeney, J., and Ridgway, S. 1975. Procedures for the clinical management of small cetaceans. J. Am. Med. Assoc. 167:540.

Thiele, D.I. and Lipsky, P.E. 1982. The accessory function of phagocytic cells in human T cell and C cell responses. *J. Immunol.* 129:1033.

Tizard, I. 1986. Basic immunology: the indispensable lymphocyte. *Vet. Med.* April:332-346.

Tizard, I. 1992. The helper T cell response. In: *Veterinary Immunology*. Saunders, Philadelphia, Pa. pp. 85-96.

Tomar, R.H. 1979. Gammopathies, hypersensitivity and immunologic deficiency. In: Tomar, R.H. (ed.). *Clinical Diagnosis and Management by Laboratory Methods*. W.B. Saunders, Philadelphia, Pa. pp. 1396-1399.

Tompkins, M.B., Gebhard, D.H., Bingham, H.R., Hamilton, M.J., Davis, W. C. and Tompkins, W.A. 1990. Characterization of monoclonal antibodies to feline T lymphocytes and their use in the analysis of lymphocyte tissue distribution in the cat. *Vet. Immunol. Immunopath.* 26:305-317.

Tompkins, M.B., Nelson, P.D., English, R.V. and Novotney C. 1991. Early events in feline retrovirus infections. *Jour. Amer. Vet. Med. Assoc.* 10:1311-1315.

United States Fish and Wildlife Service. 1989. Florida Manatee Recovery Plan. Prepared by the Florida Manatee Recovery Team for the United States Fish and Wildlife Service. Atlanta, Ga. 98 pp.

United States Fish and Wildlife Service. 1994. Florida Manatee Recovery Plan (second revision). Prepared by the Florida Manatee Recovery Team for the United States Fish and Wildlife Service. Atlanta, Ga. 176 pp.

Upton, S., Odell, D., Bossart, G. and Walsh, M. 1989. Description of two new species of *Eimeria* from the Florida manatee. *J. Protozool.* 36(1):87-90.

Vail, D.M., Mahler, P.A. and Soergel, S.A. 1995. Differential cell analysis and phenotypic subtyping of lymphocytes in bronchoalveolar lavage fluid from clinically normal dogs. *Am. J. Vet. Res.* 56:282-285.

Van Meter, V. 1987. The West Indian Manatee in Florida. Florida Power and Light Co., Miami, Fl. 39 pp.

Visser, I.K. 1993. Morbillivirus Infections in Seals, Dolphins, and Porpoises. University of Utrecht. The Netherlands. pp. 1-25.

Visser, I.K., van Bresseem, M.F., van de Bilt, Groen, J., Raga, J.A. and Osterhaus, A. 1993. Prevalence of morbilliviruses among pinniped and cetacean species. *Revue Scientifique et Technique*. 12:197-202.

Walker, C., Bode, E., Boer, L., Hansel, T.T., Blaser, K. and Virchow, J.C. 1992. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am. Rev. Respir. Dis.* 146:109-115.

Walsh, M., Bossart, G., Young, W. and Rose, P. 1987. Omphalitis and peritonitis in a West Indian manatee. *Jour. Wildlife Dis.* 23(4):702-704.

Walsh, M., Beusse, D., Bossart, G., Young, W., Odell, D. and Patton, D. 1988. Ray encounters as a mortality factor in Atlantic bottlenose dolphins. *Marine Mam. Sci.* 4(2):154-162.

Weldon, A.R., Zhang, C., Antczak, D.F. and Rebhun, W.C. 1992. Selective IgM deficiency and abnormal B cell response in a foal. *Jour. Am. Vet. Med. Assoc.* 9:1396-1398

Wells, R. and Scott, M. In press. Estimating bottlenose dolphin population parameters from individual identification and capture-release techniques. Non-lethal Techniques for Cetacean Population Estimation. International Whaling Commission.

Westermann, S. and Pabst, R. 1990. Lymphocyte subsets in the blood: A diagnostic window on the lymphoid system. *Immuno. Today*. 11 (11), 406-410.

White, J., Harkness, D., Issacks, R. and Duffield, D. 1976. Some studies on blood of the Florida manatee. *Comp. Biochem. Physiol.* 55A:413-417.

Wright, S. 1990. Personal communication. Florida Department of Natural Resources.

Yednock, T.A. and Rosen, S.D. 1989. Lymphocyte homing [Review]. *Adv. Immunol.* 44:313-378.

APPENDICES

APPENDIX A
HEMATOLOGIC
AND HISTOPATHOLOGIC
TABLES

Table 3.511
Comparison of blood count status in clinically normal dolphins

Category	N	Stat- istic	Total WBC (cells/ mm ³)	Neutro- phils (% Cells/ mm ³)	Total Lymph- ocytes (% Cells/ mm ³)	Th Lymph- ocytes (% Cells/ mm ³)	T vs Lymph- ocytes (% Cells/ mm ³)	Th: T vs	B Lymph- ocytes (% Cells/ mm ³)	NK Cells (% Cells/ mm ³)	Endo- phils (% Cells/ mm ³)	Mon- ocytes (% Cells/ mm ³)
Cb III	9	MEAN MIN MAX SD	5900 4000 7000 1157	67.3815 275.2 4851 727	22.1267 700 2212 543	83.1040 600 1013 428	11.137 0 536 167	7.7	6.75 0 219 82	0.5 0 21 9	8.436 0 770 220	2.125 0 360 145
Cc III	25	MEAN MIN MAX SD	6674 3000 9540 1864	63.4210 1290 6942 1421	19.1308 316 2886 575	73.953 202 2684 572	18.239 0 867 302	4	5.71 0 267 86	4.46 0 417 85	14.927 0 2764 749	3.228 0 728 189
Fs III	9	MEAN MIN MAX SD	5111 3000 7000 1054	66.1379 2560 4600 646	21.1049 600 1740 336	73.765 280 1556 353	21.222 48 416 140	3.4	2.25 0 96 17	4.37 0 250 81	7.364 80 770 244	6.313 160 560 147
Ff III	10	MEAN MIN MAX SD	9187 8000 10600 758	50.4260 3128 5340 714	22.2030 1229 3645 855	89.1814 1229 3280 762	8.168 0 518 173	10.7	1.30 0 81 32	1.18 0 88 27	29.2641 1367 3994 954	3.273 174 366 80

Category codes

Health status

III Clinically normal animal/normal

complete blood count (CBC)

Living environmental status

Cb Captive born animals

Cc Captive captured animals

Fs Free-ranging stranded animals

Ff Free-ranging non-stranded animals

Table 3.512
Comparison of living environmental status to acutely ill dolphins with probable bacterial infections

Category	N	Stat- istic	Total WBC counts	Neutro- phils % Cells/ mm ³	Total Lymph- ocytes % Cells/ mm ³	Tb Lymph- ocytes % Cells/ mm ³	Tc/L Lymph- ocytes % Cells/ mm ³	Thc T cells Ratio	Tb Lymph- ocytes % Cells/ mm ³	NK Cells % Cells/ mm ³	Eosino- phils % Cells/ mm ³	Adeno- cytes % Cells/ mm ³
Cb H21	9	MEAN	14940	68.10239	17.2615	67.1756	24.613	2.8	7.193	2.49	10.1517	3.411
		MIN	10000	3190	889	120	53		0	0	0	0
		MAX	33688	27120	5763	5648	289		1022	306	4800	105
		SD	5784	5061	1509	1393	74		315	101	1467	1017
Cc H21	25	MEAN	14183	72.10353	13.1859	72.1342	18.338	3.9	7.126	3.53	11.1634	3.452
		MIN	8000	4060	291	114	28		0	0	0	0
		MAX	29600	25456	4160	3564	956		1610	344	5684	1572
		SD	5311	5496	1159	1045	284		140	88	1539	361
Fs H21	9	MEAN	12144	73.8916	13.1519	83.1266	15.223	5.6	1.16	1.14	8.976	5.594
		MIN	9000	4770	475	356	0		0	0	0	0
		MAX	17400	13346	2457	2088	540		62	56	1890	1341
		SD	3908	2871	562	499	266		26	22	708	498
Ft H21	10	MEAN	15153	42.6439	20.3115	87.2712	6.197	13.8	5.154	2.53	34.5157	3.433
		MIN	12220	5255	1833	1686	59		37	29	4532	367
		MAX	19910	8760	4579	3663	458		366	92	6172	533
		SD	4157	2002	1382	991	226		184	34	887	88

Category codes
Health status
H21 Acute clinical illness with a duration of less than 8 weeks/elevated white blood count (WBC counts)
Living environmental status
Cb Captive born animals
Cc Captive-captured animals
Fs Free-ranging stranded animals
Ft Free-ranging non-stranded animals

Table 3.51.3
Comparison of living environment status in acutely ill dolphins with terminal septicemia and/or viral infection

Category	N	Statistical	Total WBC counts	Neutrophils	Total Lymphocytes	Th Lymphocytes	T c/s Lymphocyte	Th: T c/s Ratio	B Lymphocytes	NK Cells	Eosinophils	Mono-cytes
			Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³		% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³
Ch1122	no	sample										
Cc1122	5	MEAN	2825	71	17	44	35	1.2	14	6	9	3
		MIN	2000	1120	138	30	1		12	0	23	0
		MAX	4000	2320	840	504	360		109	120	720	150
		SD	888	528	293	207	154		43	56	286	65
Fs1122	2	MEAN	3000	76	18	70	30	2.3	0	0	5	6
		MIN	3000	1950	270	108	0		0	0	0	120
		MAX	3000	2610	660	660	162		0	0	150	240
		SD	0	467	276	390	115		0	0	106	85
Ff1122	No	Sample										

Category codes

Health status

1122-Acute clinical illness with a duration of less than 8 weeks/depressed white blood count (WBC count)

Living environmental status

Ch-Captive-born animals

Cc-Captive-captured animals

Fs-Free-ranging stranded animals

Ff-Free-ranging non-stranded animals

Table 3.51.4
Comparison of living environment status in chronically ill dolphins

Category	N	Stat	Total WBC counts	Neutrophils	Total Lymphocytes	Tb Lymphocytes	Tc's Lymphocytes	Tb Tc's Ratio	B Lymphocytes	NK Cells	Eosinophils	Monocytes
			Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³		% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³
Ch III	8	MEAN	10675	76	1538	81	1259	6	4	71	8	2
		MIN	5200	2912	896	758	0	0	0	0	128	0
		MAX	20500	15394	2665	1799	746		426	0	2050	537
		SD	5616	4718	567	368	286		147	0	651	192
Ch III	10	MEAN	9890	57	1641	61	1008	2.2	9	158	2	3
		MIN	4200	1722	798	435	17		0	0	1340	0
		MAX	16000	9680	3366	2424	1456		870	109	3393	1280
		SD	3991	2704	770	563	461		256	39	816	398
Ps III	No	Sample										
Pf III	No	Sample										

Category codes

Health status
III Chronic illness with a duration greater than 8 weeks/HC normal or abnormal

Living environmental status

Cb-Captive-born animals
Cc-Captive-captured animals
Fs-Free-ranging stranded animals
Ff-Free-ranging non-stranded animals

Table 3.5/5
Comparison of living environment status in dolphins under altered physiologic conditions

Category	N	Statistic	Total White counts (cells/mm ³)	Neutrophils (% cells/mm ³)	Total Lymphocytes (% cells/mm ³)	Th lymphocytes (% cells/mm ³)	T c/s lymphocytes (% cells/mm ³)	Th: T c/s Ratio	B lymphocytes (% cells/mm ³)	NK Cells (% cells/mm ³)	Eosinophils (% cells/mm ³)	Monocytes (% cells/mm ³)
Cb H4	16	MEAN	6200	60	25	73	14	5	12	1	13	1
		MIN	4700	1430	846	223	8		0	0	94	0
		MAX	8500	5950	3100	2999	558		1784	95	1404	328
		SD	1189	3206	646	723	176		441	29	343	99
Cc H4	6	MEAN	5683	65	19	71	21	3.4	8	1	12	3
		MIN	3800	2516	451	90	0		0	0	123	0
		MAX	8600	5676	2236	2246	665		271	23	1748	350
		SD	1958	3103	629	807	251		97	9	565	184
F3 H4	20	MEAN	6250	64	21	73	25	3	1	1	9	6
		MIN	4000	2520	480	134	0		0	0	0	0
		MAX	8000	5280	3626	2176	1269		65	145	1760	880
		SD	1390	935	647	453	331		22	36	430	246
FT H4	5	MEAN	9014	43	21	95	4	23.7	0	0.2	33	3
		MIN	8530	3302	1621	1621	0		0	0	2517	180
		MAX	9370	4405	2045	1999	245		36	20	3689	399
		SD	563	487	170	149	102		15	9	478	88

Category codes
Health status
H4 Physiologic conditions/ 'CW' normal
Living environmental status
Cb-Captive born animals
Cc-Captive captured animals
F3-Free ranging stranded animals
FT-Free ranging non-stranded animals

Table 3.521
Comparison of sex status vs. health status in dolphins

Core- guy	N	Stat- istic	Total White counts	Neutro- phils % Cells/ mm ³	Total Lympho- cytes % Cells/ mm ³	Th Lympho- cytes % Cells/ mm ³	T's Lympho- cytes % Cells/ mm ³	Th: T's Ratio	B Lympho- cytes % Cells/ mm ³	NK Cells % Cells/ mm ³	Eosino- phils % Cells/ mm ³	Mono- cytes % Cells/ mm ³
Male H1	27	MEAN MIN MAX SD	6011 4000 9500 1011	59 4074 2560 1014	21 1423 316 757	81 3148 202 3280 732	14 203 0 53 141	5.6	3 49 0 267 72	2 23 0 250 50	17 1153 0 3824 1022	3 279 0 738 180
Female H1	26	MEAN MIN MAX SD	6484 3000 11680 3010	61 3017 1280 6342 1752	21 1363 390 2886 574	77 1050 203 2684 570	15 207 0 867 221	5	5 64 0 257 74	3 42 0 417 84	15 987 0 3994 1067	3 186 0 468 118
Male H12	16	MEAN MIN MAX SD	13063 10000 21400 3615	63 8816 3190 37144 3464	15 2118 612 4579 1265	78 1673 128 3846 3099	17 350 53 1195 285	4.8	3 55 0 366 98	2 40 0 199 68	18 2565 0 6172 1825	3 439 0 1341 318
Female H12	39	MEAN MIN MAX SD	14500 8000 31000 5669	72 10186 4620 27120 5353	15 2104 201 5763 1373	70 1531 114 5648 1173	20 446 0 2892 582	3.4	8 168 0 1610 338	2 48 0 344 92	10 1366 0 4766 1398	3 467 0 1572 346
Male H12	2	MEAN MIN MAX SD	3500 3000 4000 707	65 2135 1050 2120 262	22 750 660 840 127	100 582 584 660 110	0 301 0 202 143	0	0 55 0 109 77	0 13 0 25 18	5 415 150 720 403	8 180 120 240 85
Male H3	7	MEAN MIN MAX SD	8600 5200 31000 1810	62 5149 2712 9600 2190	16 1363 896 2080 444	71 966 520 1456 280	23 319 0 1456 539	3	5 64 0 183 70	1 15 0 91 34	17 1437 0 3164 1309	5 451 134 1280 422
Female H3	11	MEAN MIN MAX SD	33282 4200 20500 3980	68 7647 1722 15194 3413	15 1743 798 3366 763	70 1217 435 2424 578	20 351 17 774 314	3.4	8 155 0 870 267	2 18 0 109 33	15 1686 0 3393 844	2 205 0 517 225
Male H4	15	MEAN MIN MAX SD	5780 4000 8000 1121	59 3418 1430 4060 901	27 1532 650 3626 874	76 1161 223 2999 772	20 306 9 1269 318	3.7	3 45 0 335 94	1 21 0 145 41	10 598 0 1080 341	5 221 0 720 262

Table 3.521 continued

Category	N	Stat. test	Total WBC counts	Neutrophils	Total Lymphocytes	Th Lymphocytes	T c/s Lymphocytes	Th: T c/s Ratio	B Lymphocytes	NK Cells	Eosinophils	Mono-cytes
			Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³		% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³
Female	32	MEAN	6173	60	29	76	16	4.6	7	1	16	4
HD		MIN	3809	25.6	431	90	0		0	0	0	0
		MAX	9970	59.0	2623	2236	720		1784	93	5689	880
		SD	1693	10.3	493	566	230		316	22	915	218

Category codes

Health status

HD = human normal/normal complete blood count (CBC)

HD1 = human normal/normal complete blood count (CBC)

HD2 = Acute clinical illness with a duration of less than 8 weeks/elevated WBC counts

HD3 = Acute clinical illness with a duration of less than 8 weeks/suppressed WBC counts

HD4 = Chronic clinical illness with a duration greater than 8 weeks/normal or elevated

HD5 = Physiological conditions

Table 3.522
Comparison of age vs. health status in dolphins

Cate- gory	N	Stat- istic	Total White counts	Neutro- phils % Cells/ mm ³	Total Lymph- ocytes % Cells/ mm ³	Th Lymph- ocytes % Cells/ mm ³	T cells Lymph- ocytes % Cells/ mm ³	Th. L y/f	B Lymph- ocytes % Cells/ mm ³	NK Cells % Cells/ mm ³	Eosino- phils % Cells/ mm ³	Mon- ocytes % Cells/ mm ³
A1111	1		3800	62 6472	5 390	52 203	40 176	1.3	1 27	1 4	0 0	1 468
A2111	34	MEAN MIN MAX SD	6228 4080 19830 1874	59 4076 2860 6162 962	21 1489 6400 3645 684	78 1160 280 3280 696	15 226 0 862 193	5.1	4 63 0 252 77	3 39 0 417 84	16 1098 0 3994 1090	3 237 0 728 177
A3111	32	MEAN MIN MAX SD	6258 3040 9539 2161	59 3689 1290 5925 1233	29 1269 316 2886 603	81 1035 202 2684 554	13 163 0 548 171	4.5	3 44 0 267 66	2 21 0 88 26	17 1079 0 2764 958	3 215 0 540 142
A11121	5	MEAN MIN MAX SD	14820 10080 17500 3318	63 9377 5400 12325 2674	16 2167 1200 3984 1194	79 1875 936 2918 849	16 385 64 1195 420	4.9	1 29 0 65 30	3 79 0 199 108	16 2435 0 4860 1692	4 532 166 818 297
A21121	17	MEAN MIN MAX SD	13416 8000 23000 3676	67 8993 3190 16033 3665	17 2266 291 5670 1613	68 1541 260 3967 1126	24 555 0 2892 768	2.7	5 131 0 1022 258	2 40 0 263 76	10 1341 0 5684 1607	4 523 0 1218 287
A31121	32	MEAN MIN MAX SD	14680 9000 33000 6015	71 10372 4700 23120 5295	14 2012 456 5763 1206	74 1506 114 5648 1216	16 332 28 1071 268	4.5	7 152 0 1610 380	2 43 0 344 86	12 1754 0 6172 1597	3 410 0 1572 385
A11122	No sample											
A21122	4	MEAN MIN MAX SD	3225 2000 4000 931	68 2186 1120 3354 926	19 617 460 840 172	67 411 203 660 210	22 137 0 254 111	3	10 62 0 109 49	1 7 0 25 12	9 298 0 720 311	4 125 39 240 84
A31122	3	MEAN MIN MAX SD	2767 2300 3000 404	80 2213 1890 2610 366	12 316 118 640 238	26 87 30 124 50	52 174 1 360 180	0.5	10 34 0 90 49	12 40 0 120 69	5 128 0 360 202	5 90 0 150 79

Table 3-522 - continued

Case group	N	Stat. code	Total WBC counts Cells/ mm ³	Neutro- phils % Cells/ mm ³	Total Lymph- ocytes % Cells/ mm ³	Th Lymph- ocytes % Cells/ mm ³	T % Lymph- ocytes % Cells/ mm ³	Th: T % Ratio	H Lymph- ocytes % Cells/ mm ³	NK Cells % Cells/ mm ³	Eosino- phils % Cells/ mm ³	Mono- cytes % Cells/ mm ³
A1 H3	No sample											
A2 H3	4	MEAN MIN MAX SD	10890 7000 15300 4273	59 6318 3480 9133 3063	21 2227 1540 3166 803	54 1290 415 2424 854	29 644 493 774 145	1.9	14 316 77 870 372	3 66 31 109 40	18 1918 1387 2418 532	3 338 146 490 148
A3 H3	14	MEAN MIN MAX SD	10879 4300 20500 4886	68 6878 3222 15304 4110	14 1415 798 2665 529	77 1096 520 1799 378	18 252 0 1456 411	4.3	4 63 0 426 112	0.2 3 0 30 8	15 1496 128 3393 1119	3 290 0 1280 368
A1 H4	1		8300	49 4018	21 2624	10 262	20 525	0.5	68 1784	2 0	18 1280	3 328
A2 H4	20	MEAN MIN MAX SD	5591 3840 8000 1697	59 3237 1430 5040 839	27 1529 608 3626 754	75 1147 171 2999 710	20 316 0 1269 321	3.6	3 47 0 315 81	1 19 0 145 38	10 553 0 1200 349	3 212 0 720 207
A3 H4	26	MEAN MIN MAX SD	7099 4101 9970 1585	61 4326 2596 5950 987	18 1286 451 2236 472	81 1018 540 2236 569	15 187 0 720 196	5.6	3 44 0 286 81	1 7 0 95 20	17 1220 0 3689 988	4 290 0 880 249

Category codes

Health status

H4: Physiological conditions/CBC normal

Living environmental status

Cb: Captive-born animals

Cc: Captive-captured animals

F3: Free-ranging stranded animals

F4: Free-ranging non-stranded animals

Age status

A1: ≤ 2 years of age

A2: 2-10 years of age

A3: ≥ 10 years of age

Table 3.531
Comparison of living environment status to clinically normal inmates

Core- gory	N	Stat- istic	Total Wbc count	Neutro- phils	Total Lymph- ocytes	Th Lymph- ocytes	T c/s Lymph- ocytes	Th: T c/s	B Lymph- ocytes	NK Cells	Eosino- phils	Mon- ocytes
			Cells/ mm ³	% Cells/ mm ³	% Cells/ mm ³	% Cells/ mm ³	% Cells/ mm ³	Ratio	% Cells/ mm ³	% Cells/ mm ³	% Cells/ mm ³	% Cells/ mm ³
Cb III	8	MEAN	10905	56 6112	39 4256	84 3593	11 471	7.6	3 116	2 76	1 66	4 442
		MIN	6500	4214	2072	1600	59		0	0	0	0
		MAX	17880	9620	6970	5821	1952		444	241	292	980
		SD	3991	2039	1942	1833	628		152	80	109	365
Cc III	2	MEAN	7800	59 4300	35 2561	53 1353	29 741	1.9	11 284	7 183	4 147	4 268
		MIN	4800	3504	1104	497	276		166	166	0	144
		MAX	9800	5006	4018	2210	1205		402	201	294	392
		SD	3536	1126	2661	1211	657		167	25	208	175
Fs III	18	MEAN	9706	52 5021	44 4290	89 3805	7 312	12.2	2 89	2 83	1 17	3 378
		MIN	5800	2608	1740	1472	52		0	0	0	0
		MAX	13880	7564	6486	6162	1264		275	420	303	1220
		SD	2118	1508	1621	1557	300		76	112	71	339

Category Codes

Health Status

III: Clinically normal animal/normal complete blood count (CBC)

Living environmental status

Cb: Captive-born animals

Cc: Captive-captured animals

Fs: Free-ranging stranded/injured animals

Table 3.532
Comparison of living environment status in mostly ill inmates with penitentiary bacterial infections

Category	N	Stat- istic	Total WBC counts (cells/ mm ³)	Neutro- phils (% Cells/ mm ³)	Total lympho- cytes (% Cells/ mm ³)	Th lympho- cytes (% Cells/ mm ³)	T c/a lympho- cytes (% Cells/ mm ³)	Th/ T c/a Ratio	B lympho- cytes (% Cells/ mm ³)	NK Cells (% Cells/ mm ³)	Eosino- phils (% Cells/ mm ³)	Mono- cytes (% Cells/ mm ³)
Ch H21	1		15300	60	40 6120	92 5640	4 245	23	4 245	0	0	0
Cc H21	No sample											
Ps H21	9	MEAN MIN MAX SD	17713 12800 24800 4012	54 9688 4408 15624 3074	40 7138 2432 9880 2181	78 5554 2164 8102 2131	14 1006 122 4566 1185	5.5	2 98 0 190 73	6 479 0 2473 831	1 21 0 170 60	5 865 170 1800 630

Category Codes

Healthy Status

H21: Acute clinical illness with a duration of less than 8 weeks/
elevated white blood count (WBC counts)

Living environmental status

Ch: Captive born animals

Cc: Captive captured animals

Ps: Free-ranging stranded/injured animals

Table 3.533
Comparison of living environment status in acutely ill inmates with terminal septicemia and/or viral infection

Category	N	Stat- istic	Total WBC counts (cells/ mm ³)	Neutro- phils (% Cells/ mm ³)	Total lympho- cytes (% Cells/ mm ³)	Th lympho- cytes (% Cells/ mm ³)	T c/a lympho- cytes (% Cells/ mm ³)	Th/ T c/a Ratio	B lympho- cytes (% Cells/ mm ³)	NK Cells (% Cells/ mm ³)	Eosino- phils (% Cells/ mm ³)	Mono- cytes (% Cells/ mm ³)
Ch H22	No sample											
Cc H22	No sample											
Ps H22	1		7600	58 4408	40 3040	82 2492.8	12 364.8	6.8	1 30.4	5 152	0 0	2 152

Category Codes

H22: Acute clinical illness with a duration of less than 8 weeks/
depressed white blood count (WBC counts)

Living environmental status

Ch: Captive born animals

Cc: Captive captured animals

Ps: Free-ranging stranded/injured animals

Table 3.534
Comparison of living environment status to monkeys under altered physiological conditions

Category	N	Statistic	Total WBC counts	Neutrophils	Total lymphocytes	Th lymphocytes	T c/s lymphocytes	Th: T c/s Ratio	B lymphocytes	NK Cells	Eosinophils	Monocytes							
			(cells/mm ³)	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³		% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³							
Cb-114	1		9883	41	52	5148	91	4819	4	286	23.4	2	101	0	0	4	396		
Cs-114	No	sample																	
Ps-114	16	MEAN	9124	75	21	2017	81	1640	12	248	6.6	6	110	1	20	0	18	4	365
		SD	5086	3259	648	421	421	421	21	21		0	0	0	0	0	0	0	0
		MIN	1200	1103	4937	4542	4542	1068	605	202							290	1080	
		MAX	1938	3071	1057	1028	1028	267	151	52							73	323	

Category Codes

Health Status

1122 Acute clinical illness with a duration of less than 8 weeks/
depressed white blood count (WBC counts)

Living environmental status

Cb: Active born animals

Cs: Captive captured animals

Ps: Free ranging stranded/injured animals

Table 3.541
Comparison of sex status vs. health status in manatees

Category	N	Stat- istic	Total WBC counts	Neu- phils % (cells/ mm ³)	Total Lymph- ocytes % (cells/ mm ³)	Th Lymph- ocytes % (cells/ mm ³)	T of Lymph- ocytes cells % (cells/ mm ³)	Th T cells Ratio	B Lymph- ocytes % (cells/ mm ³)	NK Cells % Cells/ mm ³	Eosino- phils % Cells/ mm ³	Mon- ocytes % Cells/ mm ³
Male 111	11	MEAN MIN MAX SD	19844 6590 17880 3231	52 5682 1131 9690 1946	43 4649 2972 6370 1226	86 4021 1616 6141 1206	9 417 59 1952 524	9.5	2 89 0 402 116	3 102 0 241 81	1 61 0 303 119	4 406 65 980 209
Female 111	17	MEAN MIN MAX SD	9276 4800 13800 2519	54 5032 2698 7564 1526	41 3838 1164 6486 1208	85 3278 497 6162 1663	9 156 52 1264 312	9.4	3 125 0 444 115	3 80 0 420 114	1 27 0 292 79	4 377 0 1220 361
Male 1121	1		15200	29 4408	66 9880	81 8102	12 1186	6.8	1 99	6 494	0 0	6 942
Female 1123	8	MEAN MIN MAX SD	17913 14900 23000 3708	55 9821 5285 15624 1669	41 7347 5912 9513 1446	80 5849 2281 7670 1751	13 972 215 4566 1375	6	2 124 0 245 83	5 402 0 2473 855	1 21 0 170 60	3 721 0 1809 722
Male 1122	No Sample											
Female 1122	No Sample											
Male 114	7	MEAN MIN MAX SD	8311 5980 10100 1675	66 5462 4259 7452 1431	30 2567 648 5148 1869	89 3288 421 4839 1795	7 175 49 346 115	13	3 98 21 242 80	1 6 0 42 16	1 41 0 299 110	2 233 0 560 215
Female 114	11	MEAN MIN MAX SD	9982 7400 12700 1903	76 7582 5025 11303 2149	20 1958 952 3080 588	78 1526 706 2640 607	15 194 23 1808 305	5.2	6 109 0 685 176	1 29 0 202 62	0 0 0 0 0 0 0 0	3 425 0 1089 344

Category Codes

111 Clinically normal animal/normal complete blood count (CBC)

1121 Acute clinical illness with a duration of less than 8 weeks/elevated white blood count (WBC count)

1122 Acute clinical illness with a duration of less than 8 weeks/depressed WBC count

113 Chronic clinical illness with a duration greater than 8 weeks/CBC normal or abnormal

114 Physiological conditions

Table 3.542
Comparison of age vs. health status by parameters

Code group	N	Stat. title	Total White counts Cells/mm ³	Neutrophils % Cells/mm ³	Total lymphocytes % Cells/mm ³	Th lymphocytes % Cells/mm ³	Tc/Lymphocytes % Cells/mm ³	Hb. Cells Ratio	B lymphocytes % Cells/mm ³	NK Cells % Cells/mm ³	Eosinophils % Cells/mm ³	Mono-cytes % Cells/mm ³
A1.011	2	MEAN MIN MAX SD	6800 6500 7100 424	38 3494 2698 1126	50 2815 2080 1039	91 2478 1726 1064	4 217 142 106	22.7	4 81 21 142 86	1 39 36 42 4	0 0 0 0	6 459 65 852 556
A2.011	17	MEAN MIN MAX SD	10944 7300 17600 2923	56 6170 4234 3489	39 4274 1840 1701	85 3639 1472 1602	11 472 59 483	7.7	2 89 0 444 109	2 74 0 241 86	1 31 0 292 80	4 468 0 1220 369
A3.011	9	MEAN MIN MAX SD	8543 4800 10500 2026	47 4017 2945 5250 935	49 4232 1104 1923	86 3681 497 6141 2008	6 266 52 1205 364	13.8	5 157 35 402 127	3 127 0 420 130	1 66 0 303 132	3 223 0 400 126
A1.021	1		15200	29 4408	65 9880	82 8102	12 1186	9.8	1 99	5 494	0 0	6 912
A2.021	7	MEAN MIN MAX SD	18129 15100 24000 3752	56 10328 5285 15624 1651	39 2223 5012 9513 1546	78 5685 2283 7670 1824	14 908 245 4666 1591	5.7	2 130 0 245 88	6 460 0 2473 907	1 24 0 170 64	4 804 0 1800 738
A3.021	2	MEAN MIN MAX SD	13550 12800 14300 1061	44 8138 6292 9084 2611	55 5149 2432 7865 3842	89 4582 2164 7000 3419	10 454 122 787 470	8.8	1 51 24 79 38	0 61 0 122 86	0 0 0 0 0	1 264 143 384 170
A1.022	No Sample											
A2.022	No Sample											
A3.022	No Sample											
A1.04	2	MEAN MIN MAX SD	11300 9900 12700 1980	44 7830 4156 11103 4912	52 3146 1143 5148 2832	94 2934 1029 4839 2694	4 132 57 206 105	23.4	1 74 46 103 40	1 6 0 11 8	0 0 0 0 0	4 325 254 396 100
A2.04	1	MEAN	9680	44 4759	51 4937	92 4541	7 346	13.1	1 49	0 0	3 290	2 194

Table 3.542 (continued)

Category	N	Statistic	Total WBC counts	Neutrophils	Total lymphocytes	Th lymphocytes	T c/s lymphocytes	Th T c/s	IL lymphocytes	NK Cells	Eosinophils	Monocytes
			cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	Ratio	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³	% Cells/mm ³
A3 H4	14	MEAN	9229	75.6959	79.1476	14.254	5.8		6.119	1.322	0	4.385
		MIN	5906	4184	421	23			0	0	0	0
		MAX	12500	10829	2666	1008			605	202	0	1080
		SD	1861	1718	717	281			160	56	0	341

Category Codes

Health Status

H1: Clinically normal animals/normal complete blood count (CBC)

H2: Acute clinical illness with a duration of less than 8 weeks/elevated white blood count (WBC counts)

H3: Chronic clinical illness with a duration of less than 8 weeks/depressed WBC counts

H4: Chronic clinical illness with a duration greater than 8 weeks/CPU normal or abnormal

H5: Physiologic conditions

Age status

A1 < 2 years of age

A2 2 - 10 years of age

A3 > 10 years of age

Table 3.551
Immunocyte Dynamics Serial Case Studies - D-diphina

Animal	Blood Date	Health Category	WBC (cells/mm ³)	Neutrophils (abs)	Total Lymphocytes (abs)	Tb (at a)	Tc/s (abs)	B (abs)	NK (abs)	Eosinophils (abs)	Monocytes (abs)
"Bebe"	10/7/91	H21	18,300	12,444	1,098	129	110	659	0	4,192	366
	10/17/91	H21	13,100	9,831	1,197	126	479	599	0	2,104	399
	10/28/91	H21	11,500	8,855	1,725	867	173	690	0	805	113
"P.J."	12/9/91	H21	19,200	10,560	3,264	2,918	65	196	196	4,800	576
	12/20/91	H21	16,600	10,458	3,984	2,540	1,195	199	199	1,992	166
	1/9/92	H21	13,800	8,142	1,704	1,333	359	0	0	3,036	828
"Aphrodite"	1/17/92	H21	10,000	5,800	1,200	934	240	0	0	2,200	800
	1/4/91	H21	12,700	8,128	1,397	1,257	28	0	112	2,794	181
	1/18/91	H21	15,500	5,215	4,030	3,546	403	40	40	3,255	0
"Misty"	7/8/91	H3	8,700	4,437	870	852	17	0	0	3,393	0
	7/20/91	H3	8,200	5,084	1,476	1,343	89	15	15	1,640	0
	12/12/91	H21	15,500	11,935	2,170	1,786	347	87	87	1,085	310
"Papi"	1/3/92	H21	9,500	6,650	1,615	1,454	162	0	0	1,235	0
	8/13/91	H21	10,500	7,350	1,680	1,092	588	0	0	1,050	420
	8/30/91	H21	10,000	7,100	1,400	840	560	0	0	1,300	200
"Papi"	9/6/91	H21	11,700	8,775	1,521	1,065	456	0	0	1,287	117
	10/21/91	H3	7,900	5,688	1,817	1,563	182	73	0	395	0
	11/2/91	H3	10,600	7,950	1,378	758	551	69	0	1,272	0
"Delta"	11/27/91	H3	20,500	15,375	2,665	1,492	746	426	0	2,050	410
	12/6/91	H3	17,900	15,394	1,253	1,163	150	0	0	716	517
	1/6/92	H3	10,200	7,140	1,836	1,800	37	0	0	1,172	102
"Papi"	8/25/89	H21	21,400	17,334	642	178	308	186	20	2,782	642
	10/10/89	H21	12,700	7,493	889	622	177	80	9	3,937	181
	1/22/90	H3	7000	4,060	1,170	840	168	101	111	1,370	490
"Delta"	2/20/90	H3	11,600	6,780	1,160	1,044	58	5	80	3,364	696
	4/21/91	H3	16,000	9,600	2,080	520	1,456	10	40	3,040	1,280
	4/5/91	H21	13,100	7,074	1,965	1,788	118	39	20	3,275	756
"Delta"	5/21/91	H21	16,000	7,840	4,160	3,120	624	41	0	3,200	800
	5/27/91	H21	13,300	7,581	2,660	2,447	133	53	27	2,527	532
	9/18/91	H3	15,300	9,333	3,366	2,424	774	137	34	2,795	306
"Delta"	10/15/91	H3	13,600	8,568	3,126	415	762	870	109	2,448	408

Table 3.55(1) -continued

Animal	Breed Date	Health Category	WBC (cells/ mm ³)	Neutrophils (abs)	Total Lympho- cytes (abs)	Ib (a-f)	T ₁ /s (abs)	R (abs)	NK (abs)	Eosino- phils (abs)	Mono- cytes (abs)
"Natus"	9/12/91	III	5,300	2,912	1,456	1,456	0	0	0	676	156
	9/20/91	III	6,400	5,120	896	896	0	0	0	128	256
	9/27/91	III	6,300	5,427	1,005	1,015	0	0	0	114	139
"Taka"	8/20/89	III	26,200	73,118	1,310	1,190	183	0	26	0	1,017
	8/25/89	III	15,200	14,116	456	111	278	23	91	0	728
	8/30/89	III	19,100	15,471	3,418	2,215	860	0	344	0	1,572
"Schwarz"	8/8/91	III	10,100	2,979	1,616	1,551	65	0	0	0	505
	10/15/91	III	15,000	13,234	1,510	1,478	0	62	0	0	610
	10/23/91	III	17,400	13,256	1,392	1,169	111	56	56	870	1,218
"Sharkey"	4/8/91	III	6,000	3,660	1,740	1,566	174	0	0	300	300
	4/15/91	III	4,000	2,360	800	728	56	16	0	80	360
	8/8/91	III	7,000	4,680	080	784	196	0	0	770	560
	9/4/91	III	5,000	3,250	1,250	625	300	74	250	300	200

abs=absolute numbers - cells/mm³

Table 3.552
Immunocyte Dynamics - Serial Case Studies - Mammals

Animal	Blood Date	Health Category	WBC (Cells/mm ³)	Neutrophils (abs)	Total Lymphocytes (abs)	Th (abs)	Tc/s (abs)	IL (abs)	NK (abs)	Eosinophils (abs)	Monocytes (abs)
"Miajory"	3/23/91	I21	15,000	5,285	9,813	2,383	4,566	100	2,473	0	302
	3/25/91	I21	17,900	11,008	5,012	3,611	101	50	50	0	1,790
	4/7/91	I21	22,500	14,400	6,300	5,670	252	189	189	0	1,800
	4/13/91	I21	16,400	8,036	7,549	7,016	177	151	0	0	820
"Avalia"	6/24/91	I14 (Pregnant)	11,000	10,829	952	876	76	0	0	0	119
	7/1/91	I14 (Pregnant)	9,000	6,710	2,570	706	1,008	605	0	0	270
	7/25/91	I14 (Pregnant)	9,000	6,920	1,710	1,505	137	34	202	0	270
	8/28/91	I14 (Pregnant)	7,500	5,025	2,250	2,003	135	113	34	0	225
"Orion"	2/19/90	I14 (Cold Stress)	8,000	6,320	1,120	806	56	168	0	0	560
	2/21/90	I14 (Cold Stress)	8,100	7,452	648	421	162	65	0	0	0
	3/21/91	I14 (Cold Stress)	5,900	4,484	1,993	923	40	40	0	0	413
"Aracile"	8/10/90	I11	8,000	5,280	2,720	2,185	326	109	0	0	0
	12/5/90	I11	9,200	7,176	1,840	1,472	368	0	0	0	184
	2/8/91	I11	9,200	5,316	3,496	3,216	210	70	0	0	368
	11/9/91	I11	8,000	5,680	2,080	1,614	416	0	0	0	240

abs=absolute numbers - cells/mm³

Table 3.621
Summary of lymph node histopathologic evaluation

	N=	NO PATHOLOGIC FINDINGS		LYMPHOID DEPLETION*		LYMPHOID HYPERPLASIA*	
		LYMPH NODES	SPLEEN	LYMPH NODES	SPLEEN	LYMPH NODES	SPLEEN
DOLPHINS	15	3	2	11	10	1	3
MANATEES	10	1	6	1	1 ^a	6	1 ^a

LEGEND

* BOTH T AND B CELL DEPENDENT ZONES UNLESS OTHERWISE INDICATED

1. B CELL DEPENDENT ZONE ONLY (1 CASE)

2. PLASMA CELL HYPERPLASIA (1 CASE)

3. T CELL DEPENDENT ZONE ONLY

4. COMBINED MILD SPLENIC B CELL DEPENDENT ZONE LYMPHOID DEPLETION WITH PLASMA CELL HYPERPLASIA IN THE SAME MANATEE

NOTE: LYMPH NODES IN 2 MANATEES (N=2) AND SPLEN IN 3 MANATEES (N=3) NOT EXAMINED

Table 3.622
Graded histopathologic lymphoid tissue results in d-diphlas**

	HEALTH CATEGORIES/ ANIMAL ID.	LYMPHOID DEPLETION *		OTHER	PRIMARY HISTOPATHOLOGIC DIAGNOSIS
		LYMPH NODES *	SPLEEN		
1	B0263, F, 3/C/0122 Epilepsy/Health Categories	4.5	4.5	Bone Marrow = NSF	Severe Chronic Active Viral Hepatitis
2	B0263, F, 3/C/0122	0	0	GALT Hyperplasia	Acute Enterocolitis
3	B0267, M, 1/C/0121	0	0	NA	Acute Enterocolitis with Hepatic Lipidosis
4	B0051, M, 1/C/0121	4.5	4.5	NA	Chronic Active Suppurative Bronchopneumonia
5	M03943, F, 2/C/0121	4.5	4.5	NA	Acute Necrohemorrhagic Bronchopneumonia (Nonbacterial with secondary bacterial infection)
6	CF92-2405, F, 3/F/0122	4.3	4.3	NA	Chronic Active Suppurative Bacterial Bronchopneumonia (Nonbacterial with secondary bacterial infection)
7	CF92-5936, F, 3/F/0122	4.5	4.5	NA	Chronic Active Fibrotic Desquamatory Pneumonia (Nonbacterial with secondary bacterial infection)
8	CF91-5190, F, 3/C/0121	4.5	4.3	NA	Acute Necrotizing Tracheobronchitis with Suppurative Mycotic Pneumonia (Nonbacterial with secondary fungal infection)

Table 3.622 - continued

	Case#(Sex/Age)/Living Environments/Health Categories	LYMPH NODES*	SUT/EN	
9	CT91-4582, 3/Cb/H21	+5	+3	NA
				Acute Necrotizing Tracheobronchitis with Suppurative Pneumonia (Morbilliviral with secondary bacterial infection)
10	CT91-3017, 2/Fs/H22	+5	+5	NA
				Acute Suppurative Mycotic Bronchopneumonia with Associated Marked Leukopenia (Morbilliviral with secondary fungal infection)
11	CT91-3312, 3/Cs/H22	+5	+3	NA
				Chronic Active Fibrotic Suppurative Bronchopneumonia (Morbilliviral infection)
12	B0101376, 2/Cb/H22	+5	0	Splenic Lymphoid Hyperplasia
				Chronic Pyogranulomatous Mycotic Bronchopneumonia (Morbilliviral with secondary fungal infection)
13	B0095733, 2/Cb/H22	+5	+1	NA
				Chronic Necrotizing Mycotic Bronchopneumonia (Morbilliviral with secondary bacterial infection)
14	B0081136, 3/Cb/NA	0	0	Atypical Lymphoid Hyperplasia (Both Spleen and Lymph nodes)
				Acute Suppurative Lymphadenitis
15	B010197X, 2/Cs/H22	0	0	Splenic Lymphoid Hyperplasia
				Acute Necro- hemorrhagic Enterocolitis

LEU-ENB

** GRADE RANGES: 0 = NONE; +1 = MILD; +3 = MODERATE; +5 = SEVERE; NE = NOT EXAMINED; NSF = NO SIGNIFICANT FINDINGS;

NA = NOT APPLICABLE; GALT = GASTROINTESTINAL-ASSOCIATED LYMPHOID TISSUE

* BOTH T AND B CELL DEPENDENT ZONES UNLESS OTHERWISE INDICATED.

1 = ≤ 1 YEARS; 2 = 2 - 10 YRS; 3 = ≥ 10 YRS.

AGE CATEGORIES

ENVIRONMENTAL CATEGORIES

HEALTH CATEGORIES: H21 = LESS THAN 8 WEEKS ACUTELY ILL WITH LEUKOCYTOSIS; H22 = LESS THAN 8 WEEKS ACUTELY ILL WITH LEUKOPENIA

FREE RANGING STRANDED

WITH

Table 3.623

Circled histopathologic lymphoid tissue results in tunanites*

HEALTH CATEGORY/ANIMAL ID	LYMPHOID HYPERPLASIA**		OTHER
CASE #/SEX ***	LYMPH. NODES	SPLEEN	
MSW 9410/F	NE	0	
MFC 9421/F	+3	0	
MSW 9410/M	+3	0	
MFC 9410/M	+1	+3	Mild Splenic B Cell Dependent Zone Lymphoid Depletion
MSF 9412/F	NE	0	
MSW 9348/F	+3	0	
MSW 9316/F	+3	NE	
MSE 9317/M	+3	0	
MFC 9408/F	0	NE	Mild Lymph Node T Cell Dependent Zone Lymphoid Depletion
MFC 9306/M	0	NE	

*THE CAUSE OF DEATH IN ALL CASES WAS RELATED TO TRAUMATICALLY INDUCED INJURIES AND HISTOPATHOLOGIC FINDINGS WERE NONSPECIFIC RELATED TO ACUTE AGONAL CARDIOVASCULAR COLLAPSE

GRADE RANGES: 0 = NONE; +1 = MILD; +3 = MODERATE; +5 = SEVERE; NE = NOT EXAMINED

**BOTH T AND B CELL DEPENDENT ZONES UNLESS OTHERWISE INDICATED

***ALL ADULTS BASED ON LENGTH (AGE UNKNOWN)

***ALL FREE-RANGING INFESTED/STRANDED (FO) ENVIRONMENTAL CATEGORY

***ALL MANATEES CONSIDERED NORMAL AND HEALTHY (HU) CATEGORY PRIOR TO DEATH

***PLASMA CELL HYPERPLASIA ONLY

***B CELL DEPENDENT ZONE HYPERPLASIA ONLY

APPENDIX B
STATISTICAL TABLES

Table 2.710

Significantly different immunocyte parameters by health category in dolphins

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H1 vs H3 H21 vs H3 H21 vs H4 H3 vs H4 H3 vs H22	49.5886	4-176	<0.001
Neutrophils H1 vs H21 H1 vs H3 H21 vs H22 H21 vs H3 H21 vs H4 H3 vs H4 H3 vs H22	36.2466	4-176	<0.001
Total Lymphocytes H1 vs H21 H21 vs H22 H21 vs H4	8.5252	4-176	<0.001
Th Cells H1 vs H21 H21 vs H4 H22 vs H21	5.3796	4-176	<0.001
Tc/s Cells H1 vs H21	2.9236	4-176	0.023
Eosinophils H21 vs H4 H21 vs H22	4.5820	4-176	0.001
Monocytes H1 vs H21 H21 vs H4 H21 vs H22	7.0088	4-176	<0.001

Table 2.720

Significantly different immunocyte parameters in captive born (Cb) dolphins

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H4	16.3419	3-49	<0.001
Neutrophils H3 vs H4 H1 vs H21 H21 vs H4	11.7259	3-49	<0.001
Total Lymphocytes H1 vs H21 H21 vs H4	4.6314	3-49	0.006
Eosinophils H1 vs H21	3.1844	3-49	0.032
Monocytes H1 vs H21 H21 vs H4	8.8961	3-49	<0.001

Table 2.730

Significantly different immunocyte parameters in captive-captured (Cc) dolphins

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H3 H21 vs H4 H21 vs H22 H3 vs H22	18.5358	4-64	<0.001
Neutrophils H1 vs H21 H21 vs H3 H21 vs H4 H21 vs H22	12.2632	4-64	<0.001
Total Lymphocytes H21 vs H22	3.1480	4-64	0.020
Eosinophils H1 vs H3 H3 vs H22	4.2533	4-64	0.004

Table 2.740

Significantly different immunocyte parameters in free-ranging stranded (Fs) dolphins

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H4	40.9636	2-35	<0.001
Neutrophils H1 vs H21 H21 vs H4	37.1318	2-35	<0.001
Eosinophils H1 vs H21	3.7513	2-35	0.033

Table 2.750

Significantly different immunocyte parameters in free-ranging (Ff) dolphins

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H4	16.6156	2-15	<0.001
Neutrophils H1 vs H21 H21 vs H4	7.7819	2-15	0.005
Eosinophils H1 vs H21 H21 vs H4	10.3945	2-15	0.002
Monocytes H1 vs H21	4.2297	2-15	0.035

Table 2.760

Significantly different immunocyte parameters in Healthy dolphins (HI) by living environment category

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) Cc vs Ff Cc vs Fs Cb vs Ff Ff vs Fs	14.1282	3-49	<0.001
Total Lymphocytes Cc vs Ff Cb vs Ff Ff vs Fs	5.0065	3-49	0.004
Th Cells Cc vs Ff Cb vs Ff Ff vs Fs	7.0132	3-49	0.001
Eosinophils Cc vs Ff Cb vs Ff Ff vs Fs	23.9427	3-49	<0.001

Table 2.770

Significantly different immunocyte parameters in acutely -ill (H21) dolphins by living environment category

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Eosinophils Cc vs Ff Cb vs Ff Ff vs Fs	7.0931	3-52	<0.001

Table 2.780

Significantly different immunocyte parameters in chronically - ill (H3) dolphins by living environment category

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
NK Cells Cc vs Cb	4.7574	1-16	0.044
Eosinophils Cc Vs Cb	15.5575	1-16	0.001

Table 2.790

Significantly different immunocyte parameters in dolphins under altered physiologic states (H4) by living environment category

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) Cc vs Ff Cb vs Ff Ff vs Fs	7.4345	3-42	0.001
Th Cells Cc vs Ff Fs vs Ff	3.4329	3-42	0.025
Eosinophils Cc Vs Ff Cb vs Ff Ff vs Fs	43.3091	3-42	<0.001
Monocytes Cc vs Ff Cb vs Fs	9.7076	3-42	<0.001

Table 2.791

Significantly different immunocyte parameters in healthy (H1) dolphins by sex

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Monocytes M vs F	4.4628	1-51	0.039

Table 2.792

Significantly different immunocyte parameters in acutely -ill (H21) dolphins by sex

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Eosinophils M vs F	7.7033	1-54	0.008

Table 2.793

Significantly different immunocyte parameters in dolphins under altered physiologic states by sex

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) M vs F	4.2360	1-45	0.045
Neutrophils M vs F	4.4610	1-45	0.040

Table 2.794

Significantly different immunocyte parameters by health category in dolphins from 2 to 10 years-of-age (A2)

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H22 H1 vs H3 H21 vs H4 H21 vs H22 H3 vs H4 H3 vs H22	24.7567	4-79	<0.001
Neutrophils H1 vs H21 H21 vs H4 H21 vs H22	18.4307	4-79	<0.001
Total Lymphocytes H1 vs H21 H21 vs H22	3.7145	4-79	0.008
B cells H1 vs H3	2.8688	4-79	0.028
Monocytes H1 vs H21 H21 vs H4 H21 vs H22	9.0475	4-79	<0.001

Table 2.795

Significantly different immunocyte parameters by health category in dolphins over 10 years-of-age (A3)

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H3 H21 vs H4 H21 vs H22 H22 vs H3	20.2447	4-85	<0.001
Neutrophils H1 vs H21 H21 vs H3 H21 vs H4 H21 vs H22	15.0363	4-85	<0.001
Total Lymphocytes H1 vs H21 H21 vs H4 H21 vs H22	6.0006	4-85	0.001
Th cells H21 vs H22	2.9374	4-85	0.025

Table 2.796

Significantly different immunocyte parameters by health category in manatees

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H4	28.6741	2-52	<0.001
Neutrophils H1 vs H21 H21 vs H4	11.7666	2-52	0.001
Total Lymphocytes H1 vs H21 H21 vs H4	26.0721	2-52	<0.001
Th Cells H1 vs H21 H1 vs H4 H21 vs H4	17.5181	2-52	<0.001
Tc/s Cells H21 vs H4	3.4176	2-52	0.040
NK Cells H1 vs H21 H21 vs H4	4.2187	2-52	0.020

Table 2.797

Significantly different immunocyte parameters by health category in free-ranging injured (Fs) manatees

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H4	33.1760	2-40	<0.001
Neutrophils H1 vs H21 H21 vs H4	10.2000	2-40	0.001
Total Lymphocytes H1 vs H21 H1 vs H4 H21 vs H4	29.9943	2-40	<0.001
Th Cells H1 vs H21 H1 vs H4 H21 vs H4	20.7750	2-40	<0.001
Tc/s Cells H1 vs H21 H21 vs H4	3.9016	2-40	0.028
NK Cells H21 vs H4	3.8585	2-40	0.029
Monocytes H21 vs H4	3.5963	2-40	0.036

Table 2.798

Significantly different immunocyte parameters by health category in manatees from 2 to 10 years-of-age (A2)

Immunocyte Parameter	F Ratio	Degrees of Freedom	P Value
Total Immunocytes (WBC) H1 vs H21 H21 vs H4	12.4515	3-32	<0.001
Neutrophils H1 vs H21	8.0361	3-32	0.001
Total Lymphocytes H1 vs H21	4.6795	3-32	0.008
B Cells H1 vs H21	8.0361	3-32	0.001
NK Cells H1 vs H21	4.6795	3-32	0.008

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PUBLICATIONS AND PRESENTATIONS

- Bossart, G.D.: A treatment regimen for candidiasis in Atlantic bottlenose dolphins. *Journal of the American Veterinary Medical Association*, 181: 1397-1401, 1982.
- Bossart, G.D.: Neurofibromas in a macaw (*Ara chloroptera*): Morphological and immunocytochemical diagnosis. *Veterinary Pathology*, 20: 733-766, 1983.
- Bossart, G.D.: A suspected acquired immunodeficiency in an Atlantic bottlenosed dolphin with lobomycosis and chronic-active hepatitis. *Journal of the American Veterinary Medical Association*, 185: 1413-1414, 1984.
- Bossart, G.D.: Ovarian papillary cystadenocarcinoma in a jaguar (*Panthera onca*). *Journal of Zoo Animal Medicine*, 14: 73-76, 1983.
- Bossart, G.D.; Odell, D.K.; Altman, N.H.: Cardiomyopathy in stranded pygmy and dwarf sperm whales. *Journal of the American Veterinary Medical Association*, 187: 1137-1140, 1985.
- Bossart, G.D.: Co-Principal Investigator, Final Report: Study of The Effects Of Oil On Marine Turtles. Prepared for the U.S. Department of the Interior, Minerals Management Service (MMS contract No. 14-120001-30063), 1986.
- Bossart, G.D.: Erysipelothrix vesicular glossitis in a killer whale (*Orcinus orca*). *Journal of Zoo Animal Medicine*, 19 (1-2): 42-47, 1988.
- Bossart, G.D.; Brawner, T.A.; Cabal, C., et al.: Hepatitis B-like infection in a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*). *Journal of the American Veterinary Medical Association*, 196 (1): 127-130, 1990.
- Bossart, G.D., and Mealey, B.K.: Bilateral anophthalmia in a screech owl (*Otus asio*). *Wildlife Rehabilitation Today*, 1 (3): 5-6, 1990.
- Bossart, G.D.: Acute necrotizing enteritis associated with suspected coronavirus infection in three harbor seals (*Phoca vitulina*). *Journal of Zoo and Wildlife Medicine*, 21 (1): 84-87, 1990.
- Bossart, G.D.: Invasive gingival squamous cell carcinoma in an aged California sea lion (*Zalophus californianus*). *Journal of Zoo and Wildlife Medicine*, 21 (1): 92-94, 1990.
- Bossart, G.D. and Dierauf, L.A.: Marine mammal clinical laboratory medicine. In: Dierauf, L. (ed). *Handbook of Marine Mammal Medicine: Health Disease and Rehabilitation*. CRC Press. Boca Raton, Florida, pp. 1-53, 1990.
- Bossart, G.D.; Odell, D.K.; Walsh, M.T.; Lynch, J.D.; Beusse, D.O.; Friday, R.; Young, G.: The histopathologic findings in a mass stranding of pilot whales (*Globicephala macrorhynchus*). In: *Marine Mammal Strandings in the United States*. NOAA/NMFS, 98: 85-90, 1991.
- Bossart, G.D.; Busbee, D.; Lahvis, G; et al. Research Perspectives for Dolphin Mortalities in North America. Environmental Protection Agency. 600/R-93/217. 1993.
- Bossart, G.D.; Lutcavage, M.; Mealey, B.; and Lutz, P.: The dermatopathologic effects of oil on loggerhead sea turtles (*Caretta caretta*). In: Frink, L. (ed.). *Wildlife and Oil Spills: Response, Research and Contingency Planning*. Sheridan Press. Hanover, Pennsylvania, pp. 180-182, 1995.
- Bossart, G.D.; Cray, C.; Solorzano, J.L; Decker, S.J.; Cornell, L.H.; and Altman, N.H.: Cutaneous papovaviral-like papillomatosis in a killer whale (*Orcinus orca*). *Marine Mammal Science*, accepted for publication, 1995.