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Effects of environmental stressors on lymphocyte proliferation in Florida manatees, *Trichechus manatus latirostris*

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Abstract

The health of many Florida manatees (*Trichechus manatus latirostris*) is adversely affected each year by exposure to cold weather or harmful algal blooms (red tide; *Karenia brevis*). Exposures can be sublethal, resulting in stressed animals that are rescued and taken to authorized facilities for rehabilitation, or lethal if exposures are prolonged or unusually severe. To investigate whether sublethal environmental exposures can impair immune function in manatees, rendering animals vulnerable to disease or death, mitogen-induced proliferation was assessed in lymphocytes from manatees exposed to cold temperatures ($N = 20$) or red tide ($N = 19$) in the wild, and compared to lymphocyte responses from healthy free-ranging manatees ($N = 32$). All animals sampled for this study were adults. Lymphocytes were stimulated in vitro with either concanavalin A (ConA) or phytohemagglutinin (PHA) and proliferation was assessed after 96 h using incorporation of the thymidine analog, bromodeoxyuridine (BrdU), into newly synthesized DNA. Proliferation of lymphocytes from manatees rescued from exposure to red tide or cold-stress was approximately one-third that of lymphocytes from healthy free-ranging manatees. To examine the direct effects of red tide toxins on lymphocyte function, mitogen-induced proliferation was assessed following co-culture of lymphocytes with *K. brevis* toxin extracts. Stimulation indices decreased with increasing toxin concentration, with a significant decrease in proliferation occurring in the presence of 400 ng red tide toxins/ml. When lymphocytes from cold-stressed manatees were co-cultured with red tide toxin extracts, proliferative responses were reduced even further, suggesting multiple stressors may have synergistic effects on immune function in manatees.

Keywords: Manatee; Lymphocyte proliferation; Immune function

1. Introduction

The Florida manatee, *Trichechus manatus latirostris*, is an endangered subspecies of the West Indian manatee, and inhabits shallow coastal areas, lagoons, and some rivers along the Florida coast. Although natural disease in manatees is uncommon (Buergelt

Abbreviations: MML, Mote Marine Laboratory; FMRI, Florida Marine Research Institute; USGS, United States Geological Survey; SI, stimulation index; BrdU, bromodeoxyuridine; PbTx, *Ptychodiscus brevis* toxin; ANOVA, analysis of variance

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and Bonde, 1983; Buergelt et al., 1984; Bossart, 2001), the health of many manatees is adversely affected each year by naturally occurring events, such as cold weather or red tide blooms, or by numerous anthropogenic factors. In the Gulf of Mexico off the coast of southwest Florida, blooms of the toxic dinoflagellate, *Karenia brevis*, are a frequent but periodic occurrence, and produce a group of polycyclic ether toxins collectively referred to as red tide toxins, or brevetoxins. Substantial numbers of mortalities associated with toxic algal blooms and/or cold weather have been documented (Bossart et al., 1998; Bossart, 1999) and have had a significant impact on the manatee population. In 1996, at least 150 manatees died in an unprecedented epizootic along approximately 80 miles of the southwestern coast of Florida, with brevetoxicosis identified as a primary component (Bossart et al., 1998). Cold-water-associated manatee mortality can occur when water temperatures drop below 21 °C for prolonged time periods (Bossart, 1999). Because the extent of cold weather and the frequency of red tide occurrences are different each year, mortalities resulting from these events vary accordingly. Even so, the Florida Fish and Wildlife Conservation Commission has attributed 11.3% of the 961 manatee deaths of known cause since 2000 to cold and 17.2% to red tide (Table 1).

Environmental stressors such as those described above can affect immunocompetence, and consequently, the ability to fight off invading toxins, viruses, and bacteria, and may ultimately result in disease or mortality (Romano et al., 2002). Evidence is accumulating that exposure to cold and/or red tide toxins can involve the immune system of manatees. For example, lymphoid lesions indicative of immunologic exhaustion and terminal opportunistic diseases have been associated with cold-related mortality in manatees (Bossart, 1999). In animals with documented brevetoxicosis, antibody staining of histochemical specimens revealed brevetoxin in lymphocytes and macrophages of the lung, liver, and secondary lymphoid tissues, with evidence of apoptosis (Bossart et al., 1998).

To date, few attempts to evaluate the immune health of manatees have been made, with published studies demonstrating impaired immune function as a result of dietary restrictions (Manire et al., 2003) and preliminary results supporting immune suppression as a possible co-factor in the etiology of papilloma virus (Bossart et al., 2002). Recently, the cloning and isolation of the manatee interleukin-2 gene has been reported (Cashman et al., 1996; Bradley and Reynolds, 2002).

An established immunological method in humans (Santos et al., 1996; Shoker et al., 1997), lymphocyte proliferation assays have been useful in assessing immune competence in several species of marine mammal, including bottlenose dolphins (*Tursiops truncatus*) (Mumford et al., 1975; Colgrove, 1978; Lahvis et al., 1993, 1995; Erickson et al., 1995; De Swart et al., 1993, 1994, 1996), killer whales (*Orcinus orca*) (Blanchard et al., 1999), and pilot whales (*Globicephala melaena*) (Mumford et al., 1975).

Lymphocyte proliferation has also been used to characterize immune dysfunction following marine mammal exposure to environmental pollutants (De Swart et al., 1995, 1996; Lahvis et al., 1993, 1995; Ross et al., 1995a,b; De Guise et al., 1996), with experimental results suggesting an association between environmental contaminants and decreased host resistance of marine mammals to pathogenic insult. In contrast, studies to examine pollutant loads

Table 1
Manatee mortalities related to cold-stress and red tide as a percentage of mortalities with known cause of death, 2000–2003

Year	Manatee mortalities (total diagnosed)[a]	Cold-related mortalities (% of total diagnosed)	Red tide-related mortalities (% of total diagnosed)
2000	204	6.9	7.4
2001	216	14.4	7.9
2002	238	7.1	15.5
2003	303	15.5	31.7
4-year total	961	11.3	17.2

Source: Marine Mammal Pathobiology Laboratory, Florida Fish and Wildlife Conservation Commission.
[a] Total mortalities minus mortalities with undetermined cause of death and unrecovered carcasses.