Veterinary Immunology and Immunopathology 133 (2010) 81–94

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Review

Immunology of whales and dolphins

Andreas Beineke [a,*], Ursula Siebert [b], Peter Wohlsein [a], Wolfgang Baumgärtner [a]

[a] Department of Pathology, University of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany
[b] Research and Technology Center Westcoast, University of Kiel, Hafentoern, 25761 Büsum, Germany

ARTICLE INFO

Article history:
Received 26 January 2009
Received in revised form 8 May 2009
Accepted 22 June 2009

Keywords:
Cetaceans
Immunology
Immune responses
Cytokines
Leukocyte subsets
Lymphoid organs
Pathology
Immunotoxicity

ABSTRACT

The increasing disease susceptibility in different whale and dolphin populations has led to speculation about a possible negative influence of environmental contaminants on the immune system and therefore on the health status of marine mammals. Despite current efforts in the immunology of marine mammals several aspects of immune functions in aquatic mammals remain unknown. However, assays for evaluating cellular immune responses, such as lymphocyte proliferation, respiratory burst as well as phagocytic and cytotoxic activity of leukocytes and humoral immune responses have been established for different cetacean species. Additionally, immunological and molecular techniques enable the detection and quantification of pro- and anti-inflammatory cytokines in lymphoid cells during inflammation or immune responses, respectively. Different T and B cell subsets as well as antigen-presenting cells can be detected by flow cytometry and immunohistochemistry. Despite great homologies between marine and terrestrial mammal lymphoid organs, some unique anatomical structures, particularly the complex lymphoepithelial laryngeal glands in cetaceans represent an adaptation to the marine environment. Additionally, physiological changes, such as age-related thymic atrophy and cystic degeneration of the "anal tonsil" of whales have to be taken into account when investigating these lymphoid structures. Systemic morbillivirus infections lead to fatalities in cetaceans associated with generalized lymphoid depletion. Similarly, chronic diseases and starvation are associated with a loss of functional lymphoid cells and decreased resistance against opportunistic infections. There is growing evidence for an immunotoxic effect of different environmental contaminants in whales and dolphins, as demonstrated in field studies. Furthermore, immunomodulatory properties of different persistent xenobiotics have been confirmed in cetacean lymphoid cells *in vitro* as well as in animal models *in vivo*. However, species-specific differences of the immune system and detoxification of xenobiotics between cetaceans and laboratory rodents have to be considered when interpreting these toxicological data for risk assessment in whales and dolphins.

Contents

* Corresponding author. Tel.: +49 0511 953 8640; fax: +49 0511 953 8675.
E-mail address: andreas.beineke@tiho-hannover.de (A. Beineke).

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doi:10.1016/j.vetimm.2009.06.019

1. Introduction

Several marine mammal populations are influenced by anthropogenic factors, such as accidental by-catches and a reduced food supply due to overfishing of waters. Furthermore, top predator cetaceans accumulate large quantities of lipophilic environmental contaminants associated with an impairment of the health status (Bruhn et al., 1999; Siebert et al., 1999). Thus, harbor porpoises (*Phocoena phocoena*) from the North and Baltic Seas, contaminated with xenobiotics, such as polychlorinated biphenyls (PCBs) and methylmercury are more frequently affected by bacterial infections and parasitism than harbor porpoises from less-polluted waters around Norway, Iceland and Greenland (Jepson et al., 1999; Wünschmann et al., 2001; Siebert et al., 2001, 2006, 2009). Although the exact cause of this disease susceptibility remains undetermined, a possible negative influence of xenobiotics on the immune system of marine mammals is currently under discussion. In addition, increased incidence of neoplastic diseases has been described in benzo[a]pyrene- and PCB-contaminated beluga whales (*Delphinapterus leucas*) from the St. Lawrence River in Canada, potentially attributed to cancerogenic effects or reduced antitumoral immune responses due to environmental factors (De Guise et al., 1995b; Martineau et al., 1999, 2002). Since 1988, devastating mass mortalities due to morbillivirus infections, associated with virus-induced immunosuppression have been observed in different marine mammal species worldwide (Kennedy, 1998; Müller et al., 2004; Wohlsein et al., 2007). Furthermore, elevated body burdens of xenobiotics were detected in striped dolphins (*Stenella coeruleoalba*) which died during the morbillivirus epidemic in the Mediterranean Sea during 1990 and 1991, leading to speculations about environmental pollution as a confounding factor in disease outbreaks (Aguilar and Borrell, 1994). Despite efforts while investigating the immunology of marine mammals in the past decades, knowledge about the cetacean immune system is still fragmentary and several aspects of immunomodulatory xenobiotics are under debate. The present communication gives an overview of the immunology of whales and dolphins with special emphasis given to evaluating immune responses, cytokine expression and immunophenotyping of leukocyte subsets as well as the morphology and pathology of lymphoid organs. Additionally, studies on the immunotoxic effect of environmental contaminants on cetaceans are reviewed.

2. Evaluation of cellular and humoral immune responses in cetaceans

2.1. Lymphocyte transformation assay

The lymphocyte transformation assay enables the qualitative and quantitative evaluation of non-specific and antigen-specific cellular immune responses. First protocols for leukocyte stimulation using whole blood samples in the common dolphin (*Delphinus delphis*), killer whale (*Orcinus orca*) and pilot whale (*Globicephala melaena*) were described in 1975 (Mumford et al., 1975). However, optimal mitogen concentrations were not determined in these surveys. Subsequently, protocols for stimulating bottlenose dolphin (*Tursiops truncatus*) leukocytes using whole blood samples have been established by Colgrove (1978). Here, concanavalin A (Con A) induces the most intense lymphocyte proliferation when compared with phytohemagglutinin (PHA) and pokeweed mitogen (PWM) *in vitro*. Although the investigation of whole blood samples reflects the actual immunological situation of peripheral blood leukocytes, inter-individual differences of white blood cell counts and the inhibitory effect of elevated blood cortisol levels during transportation or handling of animals hindered the standardization of lymphocyte transformation assays (De Guise et al., 1996b; Koopman et al., 1999; Noda et al., 2006). Therefore, techniques for the isolation and selective enrichment of leukocytes, such as density gradient centrifugation have been established in different cetacean species. Here, separated peripheral blood lymphocytes of the bottlenose dolphin are stimulated by Con A, PHA and PWM, while the B cell mitogen lipopolysaccaride (LPS) fails to induce detectable proliferation (Lahvis et al., 1993). Interestingly, age-related reduced DNA repair mechanisms in the bottlenose dolphin lead to an increasing number of genomic mutations and micronuclei in peripheral blood lymphocytes. However, DNA damages are not associated with diminished mitogen-induced cell proliferation in aged dolphins (Gauthier et al., 1999). Further, the dose–effect relationship of mitogens on isolated peripheral blood lymphocytes, thymocytes and splenocytes of the beluga whale has been investigated by De Guise et al.