Available online at www.sciencedirect.com
SCIENCE DIRECT®
Developmental and Comparative Immunology 29 (2005) 315–331
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

In vivo exposure of clearnose skates, *Raja eglanteria*, to ionizing X-radiation: acute effects on the thymus

Jennifer T. Wyffels[a,*], Cathy J. Walsh[b], Carl A. Luer[b], Ashby B. Bodine[a]

[a]*Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC 29634, USA*
[b]*Mote Marine Laboratory, Sarasota, FL 34236, USA*

Received 12 May 2004; revised 28 July 2004; accepted 12 August 2004
Available online 25 September 2004

Abstract

To investigate for the first time the effects of ionizing radiation on thymus of a representative cartilaginous fish, juvenile clearnose skates, *Raja eglanteria*, were exposed to 0–75 Gy of X-radiation and sacrificed after 12 days. Morphometrics (weight, disc width and total length) and thymus and thymic cyst area were compared to controls using ANOVA. Thymus area declined logarithmically and medullary cysts increased as a function of dose ($P \leq 0.05$). To assess thymic recovery, skates were exposed to 0, 9, 13.5 or 18 Gy of X-radiation and sacrificed when moribund or on days 10, 20, 30 and 40 post-irradiation. Complete restoration of the thymus was not achieved during the 40-day observation period, although repopulation with pro-thymocytes and partial recovery of thymic architecture were evident histologically. The observed high radiosensitivity of *R. eglanteria* thymocytes was similar to responses of other vertebrates, but recovery time was prolonged.

Keywords: *Raja eglanteria*; Elasmobranch; Thymus; Ionizing radiation; Skate; Thymic cyst; Radiation recovery; Apoptosis

1. Introduction

Fishes (Agnatha, Chondrichthyes, and Osteichthyes) are the largest and oldest group of vertebrate animals [1]. Class Chondrichthyes is a monophyletic group of cartilaginous fishes divided into two major subclasses, Elasmobranchii, sharks and batoids (rays, skates, guitarfish and sawfish) and Holocephali, chimaeras and ratfishes. Batoids comprise 56% of total elasmobranch species and include the order Rajiformes, the skates [2]. The clearnose skate, *Raja eglanteria* Bosc, is one of 136 species in family Rajidae, the largest of all chondrichthyan families [2].

Cartilaginous fishes are considered the most primitive jawed vertebrate shown to possess an adaptive immune system [3]. The earliest phylogenetic appearance of the thymus as a discrete lymphoid organ occurs in chondrichthyan fishes, in which paired thymic tissue is located dorsal to both gill

Abbreviations: ANOVA, analysis of variance; PVC, poly vinyl chloride; MHC, major histocompatibility complex; ICAM-1, intercellular adhesion molecule.

* Corresponding author. Address: Laboratory of Biology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan. Tel.: +81 561 62 8690.
E-mail address: wyffels@clemson.edu (J.T. Wyffels).

0145-305X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2004.08.005

regions [4]. In all elasmobranchs examined, the thymus develops from buds derived from a variable number of pharyngeal pouches (4–6) that become joined into one lobed mass, although not all of the buds necessarily contribute to the mature gland [5]. A cortex and medulla can be distinguished and a connective tissue capsule surrounds the gland and projects between lobules forming trabeculae. The medulla may contain epithelial cysts that are either small intracytoplasmic cavities or large complexes that border cavities filled with amorphous material or cellular debris [6]. Some reports describe structures resembling Hassal's corpuscles and thymic nurse cells in elasmobranch thymus [4,7–9]. The thymus varies considerably in the timing and amount of involution among elasmobranch species [4,10,11].

In the early 1900s, Heineke and Rudburg described the extreme sensitivity of the mammalian thymus to ionizing radiation [12,13]. Many other investigators have since investigated the effects of ionizing radiation on the thymus, documenting it as a radiosensitive organ [14–16]. Cortical, double positive thymocytes ($CD4^+$–$CD8^+$) are more sensitive to radiation than medullary, single positive thymocytes ($CD4^-$–$CD8^+$ and $CD4^+$–$CD8^-$) [17]. Stromal cells also are affected by radiation exposure and show dynamic changes in cell surface receptors during the destructive and regenerative phases of recovery [18]. Expression of cell-interaction molecules ICAM-1 and MHC class II on thymic stromal cells decreases after irradiation, impairing the ability of the stroma to interact with surviving thymocytes and repopulate the gland [19]. Increased numbers of Hassal's corpuscles after irradiation also have been reported [14,16].

The significance of the thymus in the development of a functional T cell dependent immune system combined with the phylogenetically primitive position of skates makes the thymus of these animals an interesting experimental model for detailed studies relevant to repair after chemical and physical insults. Although there are numerous studies of the histopathological changes in the thymus of mammals after radiation, relatively few reports of effects on fish thymi exist. Because the clearnose skate will breed in captivity, a constant supply of developing eggs is readily available to researchers throughout an approximately 6 month long egg-laying season. The purpose of this study was to obtain both qualitative and quantitative data on the response of elasmobranch thymi to ionizing radiation. A comparison of data from such studies in skates to the broad database for other vertebrates will lend further support to the contention that elasmobranchs are among the first vertebrates to display a functional T cell dependent immune system.

2. Materials and methods

2.1. Animal husbandry

Clearnose skate specimens were collected in near shore waters off Anna Maria Island, FL, and maintained in captivity at Mote Marine Laboratory, Sarasota, FL, under conditions previously described [20]. Eggs laid following captive breeding of wild caught adults were incubated at Mote Marine Laboratory and shipped to Clemson University, SC, before completion of the approximately 12-week long developmental period. Egg cases were placed in either of two 93 gallon aquaria with artificial recirculating seawater. Aquaria were equipped with inline Angstrom 2537® UV sterilizers (Hawaiian Marine Imports, Inc., Houston, TX), Emperor Aquatics Series #2 protein skimmers (Emperor Aquatics, Inc., Pottstown, PA) and Aquanetics AFC-1 chillers (Aquanetics Systems Inc., San Diego, CA) to maintain the water at 21 °C. Water clarifying micron filters powered by Marineland Magnum 350 canister filters (Aquaria, Inc., Moorpark, CA) circulated water through the protein skimmers, chillers, biofilters, and UV sterilizers.

Skates hatched naturally and were fed fresh frozen herring on alternating days. Water changes (20% of total volume) were performed when nitrate exceeded 100 ppm, nitrite 0.05 ppm, or ammonia 0.05 ppm. The pH was maintained in the range of 8.0–8.5 and salinity 31–35‰. Plastic mesh netting and PVC were used to form barriers to partition skates by treatment.

2.2. Explanation of dose units

The System International (SI system) of units for radiation uses the term gray (Gy) for measurement of absorbed dose. In the existing literature, radiation