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Short communication

Apoptosis in thymus of teleost fish

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ABSTRACT

The presence and distribution of apoptotic cells during thymus development and in adult were studied by in situ end-labelling of fragmented DNA in three temperate species carp (*Cyprinus carpio*), sea bass (*Dicentrarchus labrax*) and dusky grouper (*Epinephelus marginatus*) and in the adult thymus of three Antarctic species belonging to the genus *Trematomus* spp.

During thymus development some few isolated apoptotic cell (AC) firstly appeared in the central –external part of the organ (carp: 5 days ph; sea bass: 35 days ph grouper: 43 days ph). Initially the cells were isolated and then increased in number and aggregated in small groups in the outer-cortical region of the thymus larvae. The high density of apoptotic cells was observed in the junction between cortex and medulla from its appearance (border between cortex and medulla, BCM). ACs decreased in number in juveniles and adult as well as the ACs average diameter. In late juveniles and in adulthood, the apoptosis were restricted to the cortex. In Antarctic species the thymus is highly adapted to low temperature (high vascularisation to effort the circulation of glycoproteins enriched plasma and strongly compact parenchyma). The apoptosis process was more extended (4–7 fold) as compare with the thymus of temperate species, even if the distribution of ACs was similar in all examined species. Data suggested a common process of T lymphocyte negative-selection in BCM of thymus during the ontogeny. The selection process seems to be still active in adult polar fish, but restricted mainly in the cortex zone.

1. Introduction

In fish, the thymus plays an important role in T lymphocytes differentiation [1–5] and, in certain species in myelopoesis too, like in Antarctic species [6]. Three distinct regions are involved in the differentiation of the lymphocytes: the cortex (generally external to the organ and with capsular and subcapsular portions), medulla (called also inner thymus region) and the border/junction between the cortex and medulla (BCM) [3]. These regions were distinguished on the basis of cytology, histology, immune-histochemistry of its components and recently it has been functionally defined by using specific markers for T lymphocytes [TcRβ, 2,3; CD8 and CD4, 5]. The apoptosis occurs during thymus development, with the elimination of auto-reactive and damaged lymphocytes [1,7] and the biochemical process has been defined recently in fish [8]. Besides the selection of lymphocytes, the apoptosis process can be activated by upset of mechanical, chemo-physical and pathological occurrence [9]. Among the apoptosis-inducing factors, the temperature in fish could be a major responsible to DNA damage in thymocytes, due to the superficial position of the organ [6]. Thymocytes respond to the temperature stress by activating a complex DNA-damage-response pathway that includes cell-cycle arrest, the transcriptional and post-transcriptional activations of a subset of genes including those associated with DNA repair, heat shock proteins and, under some circumstances, the triggering of programmed cell death [10,11]. Very interesting models to study the temperature-induced apoptosis in fish can be the Polar species. The Antarctic species are specialized to live in polar water, an extreme environment that ranged from 1 to −2 °C. Their tissues are protected to the freeze risk by high concentration of ions in body fluids [12] and anti-freeze circulating substances, produced in the liver [13]. Assuming that polar fishes are adapted to stress exerted by low temperature, it can be interesting evaluate differences as regard the apoptosis in the thymus.

The present study is focused on a general comprehensive study of the distribution of apoptosis in thymus of developing fish species (*Cypriniformes* and *Perciformes*) and in adulthood thymus of warm and polar waters *Perciformes* species, with a novel view of

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considering the three region of the thymus: cortex, medulla and BCM.

2. Materials and methods

Larvae, juveniles and adult (18–22 months old) fish from temperate water were maintained at different water temperature in dependence to the species (23 °C for the carp; 16 °C for sea bass; 17–18 °C for the gruppy) until they were sampled. Fishes were killed with an overdose of tricaine methanesulphonate (MS222, Sigma; 1 mg ml^{-1}) and whole fish or dissected thymus was immersion-fixed in Bouin's liquid (7 h at 4 °C) washed at 4 °C in 70% alcohol. The Antarctic fish (22–26 months old) were fished anesthetized and sampled directly *in loco* and shipped in Italy after fixation in 70% alcohol.

2.1. Histology and in situ end-labelling of fragmented DNA (ISEL)

Serial transverse paraffin sections (5 μm) were dried at 37 °C and placed on 2% 3-aminopropyltriethoxysilane (Sigma Chemical Co., St Louis, U.S.A.)-coated slides. De-waxed sections were rehydrated and stained by Mallory's trichrome, May-Grünwald/Giemsa, Haematoxylin-Eosin, according to Pappenheim's method.

The ISEL procedure was previously described [1], briefly dewaxed sections were rehydrated and incubated with TE buffer (10 mM Tris, 1 mM EDTA, pH 8) containing proteinase K (larvae: 5–10 μg ml^{-1}; juveniles: 20 μg ml^{-1}, Sigma, U.S.A). Slides were incubated for 2 h at 37 °C with TDT buffer containing the DNA-labelling mixture [0.3 I.U. μl^{-1} terminal deoxynucleotidyl transferase (Promega Pharmacia Biotech, Uppsala, Sweden), 2 μM digoxigenin-11-dUTP (2′-deoxyuridine-5′-triphosphate tetralithium salt; Roche Diagnostics), 17 μM dATP (2′-deoxyadenosine-5′-triphosphate disodium salt; Pharmacia)]. Positive controls were obtained by applying deoxyribonuclease I (from bovine pancreas; Pharmacia) (1 μg ml^{-1}, dissolved in 10 mM Tris, 10 mM NaCl, 5 mM MgCl2, 25 mM KCl, 0.1 mM CaCl2, pH 7·4) for 15 min at room temperature before the DNA-labelling step. Terminal transferase was omitted from the DNA-labelling mixture in negative control sections. Slides were washed and incubated with peroxidase-conjugated sheep anti-digoxigenin serum (Fab fragment; Roche) diluted 1:200. After rinsing were incubated in

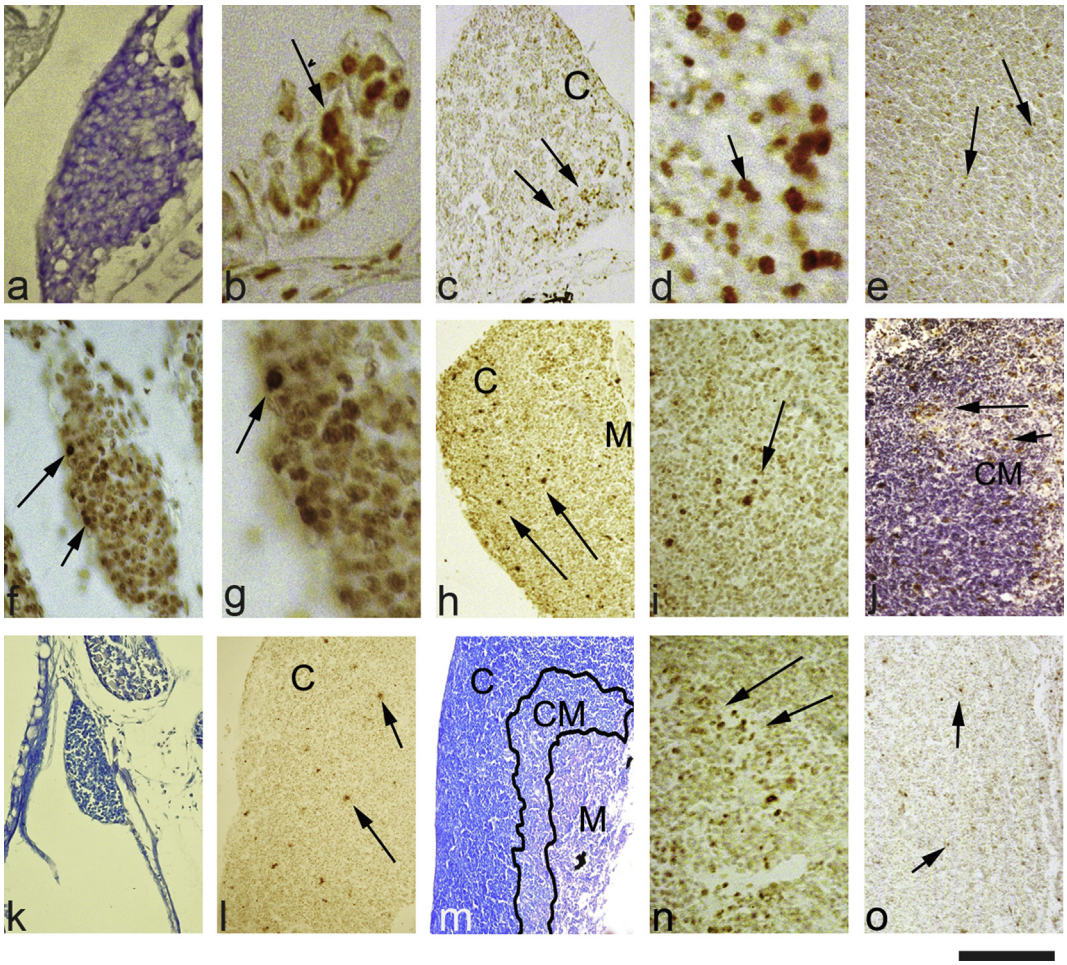

Fig. 1. Apoptosis detection by ISEL assays in developing thymus of temperate sea water fish species. (**a**–**e**) Thymus of *C. carpio* (**a**, Pappenheim'staining, bar = 40 μm); (**b**) first apoptotic cells are localized in middle-periphery region at day 7 (arrow, bar = 30 μm); (**c**) apoptotic cells are spread in the cortex and in BCM (28 d, arrows, bar = 50 μm) as singular or grouped cells (**d**, arrows; bar = 20 μm); (**e**) At 22 weeks old an extended thymus shows mainly a cortical apoptosis (arrows; bar = 50 μm). (**f**–**j**) Thymus of *D. labrax*. (**f**) At 45 d the thymus shows apoptotic cells in the periphery of the organ (arrows, bar = 40 μm), (**g**) magnification of **f**) (arrow, apoptotic cell, bar = 20 μm). (**h**) At 70 d, medulla (M), cortex (C) and BCM are clearly evident (apoptotic cells, arrows, bar = 60 μm); (**i**) in a magnification of **h**), is evident that some apoptotic cells are distributed as singular or grouped cells in BCM (arrow, bar = 35 μm). (**j**) At 120 d the BCM (Harris'staining on ISEL) shows numerous apoptotic cells (arrows, bar = 60 μm). (**k**–**o**) Thymus of *E. marginatus*. (**k**) At 20 d, the thymus is filled of lymphoid elements (Pappenhein'staining; bar = 160 μm). (**l**) At 48 d apoptotic cells (arrows) are localized mainly in the cortical region (C, bar = 60 μm). (**m**) At 78 d is demarked the BCM (CM, drawn by a dark line) that is distinguishable from cortex (C) and medulla (M) (Pappenheim'staining, bar = 80 μm). (**n**) In the same day, are show numerous apoptotic cells (arrows) mainly localized in BCM (bar = 30 μm). (**o**) At 120 d, numerous apoptotic cells are evident in cortex (arrows, bar = 40 μm).