Fish & Shellfish Immunology 27 (2009) 50–56

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Lymphocyte differentiation in sea bass thymus: *CD4* and *CD8-α* gene expression studies

Simona Picchietti [a], Laura Guerra [a], Francesco Buonocore [a], Elisa Randelli [a], Anna Maria Fausto [a], Luigi Abelli [b,*]

[a] Dipartimento Scienze Ambientali, Università della Tuscia, 01100 Viterbo, Italy
[b] Dipartimento Biologia e Evoluzione, Sezione Anatomia Comparata, Università di Ferrara, Via Borsari 46, 44100 Ferrara, Italy

ARTICLE INFO

Article history:
Received 9 July 2008
Received in revised form
21 April 2009
Accepted 23 April 2009
Available online 5 May 2009

Keywords:
Dicentrarchus labrax
Thymus
CD4
In situ hybridization
Development
Positive selection
Thymocytes

ABSTRACT

Different developmental stages (from eggs to 1-year-old juveniles) of the teleost fish *Dicentrarchus labrax* (L.) were assayed for *CD4* gene expression. RT-PCR revealed the appearance of *CD4* transcripts in post-larvae from 51 days post-hatching (dph). This finding overlaps the first detection of *CD8-α* mRNA.
Real-time PCR with specific primers quantified *CD4*, *CD8-α* and *TCR-β* transcripts in larvae and post-larvae (25, 51, 75 and 92 dph) and 1-year-old thymus. At 92 dph, *TcR-β* and *CD8-α* transcripts were significantly higher ($P < 0.001$) than in previous stages, as *CD4* transcripts compared with 51 dph ($P < 0.01$). High levels of *TCR-β* and *CD8-α* transcripts were found in the thymus, while *CD4* transcripts were lower ($P < 0.05$ vs. *TCR-β*).
In situ hybridization identified *CD4* mRNAs at 51 dph, localized in thymocytes of the outer and lateral zones of the thymic glands. From 75 dph on the signal was mainly detected in the outer region, drawing a cortex–medulla demarcation. Developmental expression of *CD4* and *CD8-α* almost coincided. In each adult thymic lobe $CD4^+$ and $CD8\text{-}\alpha^+$ thymocytes filled the cortex. The expression patterns of *CD4* and *CD8-α* largely overlap, except in the medulla, where $CD4^+$ thymocytes were isolated, while $CD8\text{-}\alpha^+$ ones mainly arranged in cords.
These results provide new information about the thymic compartmentalization and lymphocyte differentiation pathways in a teleost, almost demonstrating that double negative thymocytes fill the cortex giving rise to further selection in the medulla.

1. Introduction

The thymus in mammals provides specialized microenvironments that support and direct the differentiation and selection of T cells. The repertoire of T-cell antigen receptor (TCR) is generated by various mechanisms, including positive and negative selections, that affect primarily αβ TCR lymphocytes. Developing lymphocytes in the thymic cortex and medulla are engaged in extensive interactions with ligands of self-peptides presented by the MHC expressed on the three-dimensional network. Thus, the thymus can be viewed as an efficient filter, through which the positively selected developing T cells pass as they mature.

In teleosts no precise information is yet available on differentiation and selection of T cells, crucial steps to establishing a functional immune system, although the role of the thymic microenvironment has been recognized [1–3]. The hypothesis that T-cell subpopulations can exist in fish is supported by molecular and cell biology data, since CD4 and CD8 co-receptors were cloned in trout, fugu, ginbuna crucian carp, salmon, gilthead seabream and sea bass [4–10] and biological activities were studied [11]. Recently, two different genes were also identified in rainbow trout that resemble mammalian *CD4*: the first one (trout *CD4*) encodes four extracellular Ig domains reminiscent of mammalian *CD4*, whereas the other (*CD4REL*) encodes two Ig domains [12]. These reports open new issues about T cell development and functionality in lower vertebrates.

The present study in the European sea bass, *Dicentrarchus labrax* (L.), applied RT-PCR to investigate the developmental appearance of *CD4*. In addition, specific primers for *CD4*, *CD8-α* and *TcR-β* were used to quantify by real-time PCR gene transcripts in larvae and post-larvae and the thymus of 1-year-old specimens. *In situ* hybridization was applied to localize for the

Abbreviations: tcr, T cell antigen receptor; dph, days post-hatching; PCR, polymerase chain reaction; DP, double positive; SP, single positive; DN, double negative.

* Corresponding author. Department of Biology & Evolution, Section of Comparative Anatomy, University of Ferrara, Via Borsari 46, 44100 Ferrara, Italy. Tel.: +39 0532 291712; fax: +39 532 455715.
E-mail address: abl@unife.it (L. Abelli).

1050-4648/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2009.04.003