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The ontogeny of MHC class I expression in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

In the present study, clonal rainbow trout (*Oncorhynchus mykiss*) embryos and larvae were assayed for the expression of key molecules involved in specific cell-mediated cytotoxicity using an anti-MHC class I monoclonal Ab and by RT-PCR using specific primers derived from classical MHC class I (class Ia), TCR and CD8. Whereas RT-PCR revealed that MHC class Ia and CD8 were expressed from at least 1 week after fertilisation (p.f.) on, TCR expression was detectable from 2 weeks p.f. Immunohistochemistry indicated an early and distinct expression of MHC class I protein in the thymus. Positive lymphoid, epithelial and endothelial cells were found in the pronephros, in the spleen and in the inner and outer epithelia at later stages. Whereas in older rainbow trout the intestine is counted among the organs of the highest class I expression, during ontogeny it was the last site (39 days after hatching) where such expression was detectable. Knowledge on the appearance of the assayed key molecules during fish development is relevant for the pathogenesis of infections as well as for early vaccine delivery. Besides such information regarding the development of the adaptive immune system, immunohistochemistry revealed that in early larvae MHC class I was expressed in neurons whereas in older rainbow trout this was not observed.

Keywords: Rainbow trout (*Oncorhynchus mykiss*); Ontogeny; MHC; T cell receptor; CD8

1. Introduction

Immunocompetence is a feature acquired by the organism during ontogeny. The development of the adaptive immune system is of interest because memory responses are possible targets for vaccines

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protecting juvenile fish from disease. Considerable progress in the understanding of the development of the adaptive immune system in fish has been made. In rainbow trout, specific antibodies are transmitted from mother to embryo whereas first cytoplasmic and surface IgM positive cells can be observed 12 and 8 days (respectively) before hatching [1]. However, in the same species a first adaptive humoral immune response to a bacterial antigen can only be induced 1 month after hatching in the same species [2]. Functional studies in rainbow trout juveniles indicative of adaptive cell-mediated cytotoxicity are restricted to allograft rejection: fry as young as 14 days post-hatching can destroy skin allografts, a process accompanied by lymphocyte infiltration [3]. The thymus in fish is probably essential for the education of T lymphocytes and thus for immunocompetence. The thymus anlage was detected in rainbow trout embryos in concert with the transcriptional start of the terminal deoxynucleotidyl transferase (TdT), which has a function in T cell receptor (TCR) recombination [4]. Recombination-activating gene (RAG)1 transcription as well as that of the TCR was found in the thymus of early zebrafish (*Danio rerio*) embryos [5,6]. Other genes important in T cell function, i.e. major histocompatibility complex (MHC) class Ia and class II, are also transcribed early in fish ontogeny. However, they appear to be transcribed even before the onset of thymus development, since common carp genes of both classes are transcribed from day 1 after fertilisation [7].

The present study focuses on MHC class I expression in rainbow trout embryos and larvae, since for these early fish stages no reports on MHC class I histology are available yet. Mammalian MHC class Ia molecules are involved in offering antigenic peptides derived from endogenous proteins at the cell surface for recognition by the TCR/CD8 complex of cytotoxic T lymphocytes [8,9]. MHC class Ia molecules mediate the acquisition of a T cell repertoire by participating in the positive and negative selection of CD8 positive T cells in the thymus. The finding of rainbow trout sequence homologues for MHC class Ia [10–15], β2 microglobulin (β2m) [16], low molecular mass protein (LMP), transporter associated with antigen processing (TAP), MHC class II [13], TCR [17,18] and CD8 [19] suggests that antigen presentation in fish is similar to that in higher vertebrates. Therefore, it is not surprising that in fish cell-mediated cytotoxicity against allogeneic cells is executed by CD8 expressing lymphocytes [20] and that fish cytotoxic cells distinguish between virus-infected syngeneic and allogeneic target cells [21]. Cell-mediated cytotoxicity against virus-infected cells is probably MHC class I restricted [22]. Salmonid fish only express a single MHC class Ia locus designated *Onmy-UBA* [14,15]. Moreover, rainbow trout MHC class I molecules are expressed in similar cell types as their mammalian homologues as shown by means of a monoclonal Ab (mAb), designated H9, raised against recombinant rainbow trout MHC class Ia [23]. Thus TCR, CD8 and MHC class I seem to represent key molecules in specific cell-mediated cytotoxicity as in mammals.

The present study applied the H9 antibody for investigation of class I ontogeny. Earliest MHC class I expression was detected in the primary and secondary lymphoid tissues, in the epithelia, and in the nervous tissues. Thymic expression suggests a function in T cell selection, and expression in the secondary lymphoid tissues and epithelia should be associated with immune surveillance. The expression in nervous tissues is puzzling, but may be associated with a function of MHC class I in neuron development, as discussed for mammals [24]. In rainbow trout no antibodies are available against TCR and CD8. Therefore, in this study RT-PCR was applied to collect data on their expression at the transcriptional level.

2. Materials and methods

2.1. Fish

Eyed eggs of homozygous isogeneic rainbow trout (clone C25) were derived from the Nagano Prefectural Experimental Station of Fisheries, Nagano, Japan. The clone was produced by gynogenesis over two generations by suppression of mitosis and meiosis in the first and second generations, respectively. Clonality was confirmed by DNA fingerprinting (data not shown). For convenient propagation of the