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Review

# Perspectives on antigen presenting cells in zebrafish

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ABSTRACT

Antigen presentation is a critical step in the activation of naïve T lymphocytes. In mammals, dendritic cells (DCs), macrophages, and B lymphocytes can all function as antigen presenting cells (APCs). Although APCs have been identified in zebrafish, it is unclear if they fulfill similar roles in the initiation of adaptive immunity. Here we review the characterization of zebrafish macrophages, DCs, and B cells and evidence of their function as true APCs. Finally, we discuss the conservation of APC activity in vertebrates and the use of zebrafish to provide a new perspective on the evolution of these functions.



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## 1. Introduction

Nearly all mammalian cells are capable of presenting antigen, but three groups of cells are considered to be professional antigen presenting cells (APCs): macrophages, dendritic cells (DCs), and B cells (reviewed in Trombetta and Mellman (2005)). APCs are specialized in their ability to uptake, process, and present antigen to naïve T cells. A defining feature of APCs is their constitutive expression of major histocompatibility complex (MHC) class II molecules, which are required for the presentation of antigen to

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T cell receptors on $CD4^+$ T cells. Upon activation, APCs also express co-stimulatory molecules necessary for naïve T cell priming and secrete cytokines that direct effector T cell differentiation. Although APCs participate in innate immunity, the most significant impact of APC activity in mammals is the initiation of adaptive immune responses and subsequent acquisition of immunological memory.

Macrophages are proficient in the uptake of antigen and production of inflammatory cytokines upon stimulation, and are also important in the clearance of apoptotic cells and production of growth factors in the absence of infection. DCs were first characterized based on their distinctive branched morphology (Steinman and Cohn, 1973). In addition to key morphological differences between DCs and macrophages, DCs are unparalleled in their ability to interact with T cells in secondary lymphoid tissues, which results in either the reinforcement of T cell tolerance or activation of naïve T cells (Nussenzweig and Steinman, 1980; Steinman, 2007). Thus, DCs are considered to be the primary APC type in mammals.

B cells are developmentally and functionally distinct from the myeloid APCs. They internalize specific antigens through B cell receptor (BCR)-mediated endocytosis (reviewed in Yuseff et al. (2013)). Signaling through the BCR induces changes that facilitate antigen presentation, proliferation and affinity maturation of secreted antibodies, which results in highly specific B cells that may persist as memory B cells.

The zebrafish (*Danio rerio*) has recently emerged as a novel model system to study the immune system. In comparison to mammalian models, zebrafish are easy to genetically manipulate, highly prolific, and inexpensive to maintain in large numbers. Due to their embryonic transparency and external development, the use of zebrafish permits live visualization of developmental processes and cellular interactions without physiological disruption of tissues and organs. These features make zebrafish a potentially useful model for the study of immunological processes. In addition, the validation of zebrafish as a model to study immune responses will allow us to exploit additional powerful tools, including forward genetic and pharmacological screens.

MHC class II-expressing macrophages (Lieschke et al., 2001; Wittamer et al., 2011) and B cells (Page et al., 2013) have been characterized and demonstrated to fulfill similar roles in zebrafish as their mammalian counterparts. However, many questions about APCs in zebrafish remain unanswered. Since $CD4^+$ T cells have not been functionally characterized in zebrafish, it is unclear if the classical paradigm of MHC class II presentation persists in zebrafish. Additionally, neither lymph nodes nor other secondary lymphoid organs have been identified in zebrafish. Therefore, it remains unclear if DCs, the primary inducer of adaptive immunity in mammals, function equivalently in zebrafish. Finally, memory B and T cells have not been identified and the existence of immunological memory has not been conclusively documented in zebrafish. Thus, it remains uncertain to what extent APCs, adaptive responses, and immunological memory contribute to the overall immunity of zebrafish.

Combined with APC studies using other teleost species, including medaka, carp, cod, catfish, trout, and salmon, a clearer picture of the conserved elements of vertebrate APC ontogeny and function has emerged. This review outlines the similarities and differences between mammalian and zebrafish macrophages, DCs, and B cells, and highlights some of the outstanding questions in teleost and mammalian APC biology. In particular, we discuss the advantages of the zebrafish model for the investigation of the development and function of APCs. Finally, we place zebrafish APCs within an evolutionary context and discuss their potential use in studies of adaptive immunity.

## 2. MHC class II antigen presentation in zebrafish

MHC class II is made up of two membrane-bound glycoprotein chains, termed α and β. Humans have three classical MHC class II genes: HLA-DR, -DP, and -DQ. These genes are located within the MHC locus, which contains the tightly linked classical and non-classical MHC class I and II genes, and many other genes involved in antigen processing, antigen presentation, and immune function. Zebrafish MHC genes are located on different chromosomes, likely due to multiple translocation events that occurred in teleosts (Sambrook et al., 2005; Sato et al., 2000; Dijkstra et al., 2013). A number of MHC class II genes have been identified, but many of these appear to be pseudogenes (Sultmann et al., 1994; Dijkstra et al., 2013). Of the three MHC class II *A* loci and six MHC class II *B* loci identified (encoding α and β chains, respectively), only *mhc2dab, mhc2deb*, *mhc2daa* and *mhc2dea* contain a complete set of exons (Sultmann et al., 1994; Graser et al., 1998). A helpful chromosomal map of zebrafish MHC genes and their paralogs can be found in Sambrook et al., 2005. Notably, *mhc2dab* and human MHC class II genes contain conserved upstream regulatory sequences. Hence, although there is no single MHC locus in zebrafish, MHC class II genes and promoters are highly conserved in vertebrates.

The constitutive expression of MHC class II on APCs to be conserved in zebrafish. *Mhc2dab* transcript is abundant in the zebrafish spleen and kidney, two sites containing hematopoietic cell lineages (Wittamer et al., 2011). Furthermore, *mhc2dab* is expressed in macrophages, dendritic cells, and B cells, but not in T cells (Wittamer et al., 2011). In mammals, several additional cell types express MHC class II, including thymic epithelial cells (TECs), which mediate positive selection of developing thymic T cells (Marrack et al., 1988). Accordingly, TECs in zebrafish also express MHC class II (Wittamer et al., 2011).

MHC class II assembly occurs in the endoplasmic reticulum (ER). The α and β chains fold to form a dimer containing an open peptide-binding groove, which is bound by the membrane-anchored invariant chain. The invariant chain prevents intracellular peptides from binding the peptide-binding groove and directs the MHC class II molecule to an endosomal compartment. Proteases in endolysosomes cleave the invariant chain, leaving a peptide termed the MHC class II-associated invariant chain peptide (CLIP) in the peptide-binding groove. The non-classical MHC class II molecule HLA-DM facilitates the exchange of CLIP for an externally derived high affinity peptide (reviewed in Blum et al. (2013)). The MHC class II-associated invariant chain has been identified in teleosts (Yoder et al., 1999), however, they lack an HLA-DM homolog, suggesting an alternative mechanism for the removal of CLIP from the MHC class II peptide-binding groove (Dijkstra et al., 2013). Thus, although there is much conservation between zebrafish and mammals, certain processes are likely specific to teleosts. Further studies elucidating mechanisms of MHC class II folding, and antigen processing and presentation will uncover conserved elements and inform in what way the zebrafish can be used as an immunological model. Of note, Atlantic cod have lost their MHC class II, CD4, and invariant chain genes. However, presumably to compensate for the loss of MHC class II, the cod has greatly expanded the number of MHC class I gene loci and its innate immune receptor repertoire (Star et al., 2011).

The MHC class II–TCR interaction is crucial for the development, maintenance, activation, and maturation of $CD4^+$ T cells. During an infection, DCs present MHC class II-peptide complexes to $CD4^+$ T cells, which can result in their activation. In turn, $CD4^+$ T cells help the immune system clear pathogens by enhancing the activities of macrophages and B cells (reviewed in Blum et al. (2013), Ramiscal and Vinuesa (2013), Viret and Janeway (1999)).