Developmental and Comparative Immunology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

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

Review

Perspectives on antigen presenting cells in zebrafish

Kanako L. Lewis [1], Natasha Del Cid [1], David Traver *

Division of Biological Sciences, University of California, San Diego, La Jolla, CA, United States

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Zebrafish
Antigen-presenting cells
Dendritic cells
B lymphocytes
Macrophages

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.

Contents

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

* Corresponding author. Address: Department of Cellular and Molecular Medicine, Section of Cell and Developmental Biology, University of California, San Diego, 9500 Gilman Drive, Natural Sciences Building, Room 6107, La Jolla, CA 92093-0380, United States. Tel.: +1 (858) 822 4593; fax: +1 (858) 822 5740.
E-mail address: dtraver@ucsd.edu (D. Traver).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.dci.2014.03.010
0145-305X/

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)).

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010

Table 1
Transgenic lines available for distinguishing APC subsets in zebrafish. ("MEM" superscript indicates that fluorophore is membrane-localized.)

Regulatory region	Fluorophores	Relevant expression pattern	References
cd45/ptprc	DsRed	Most leukocytes	Bertrand et al. (2008)
rag2	eGFP, DsRed, DsRed2	Lymphocytes	Langenau et al. (2004), Langenau et al. (2007), Langenau et al. (2005)
mhc2dab	eGFP, mCherry	APCs	Wittamer et al. (2011)
mpx	eGFP, eGFP^{MEM}, Dendra2, mCherry	Neutrophils (hi), macrophages (lo)	Mathias et al. (2006), Mathias et al. (2009), Renshaw et al. (2006), Yoo and Huttenlocher (2011), Yoo et al. (2010)
lyz	eGFP, mCherry, DsRed2,TagRFP	Granulocytes, macrophages	Hall et al. (2007)
pu.1/spi1	eGFP, eGFP^{MEM}	Myeloid, lymphoid cells	Zakrzewska et al. (2010), Ward et al. (2003), Hsu et al. (2004)
mpeg	eGFP, YFP, mCherry, mCherry^{MEM}, Dendra2	Macrophages	Ellett et al. (2011), Roca and Ramakrishnan (2013), Harvie et al. (2013)
igm	eGFP	IgM$^+$ B cells	Page et al. (2013)
blimp1/prdm1a	eGFP	Plasma cells	Elworthy et al. (2008), Page et al. (2013)

However, as CD4$^+$ T cells have not been functionally characterized in zebrafish, there is still much work to be done to understand the role of MHC class II and CD4$^+$ T cells in the zebrafish adaptive immune response. Studies examining the role of zebrafish CD4$^+$ T cell help in the activation of macrophages and B cells are also needed to fully understand the evolution of the vertebrate adaptive immune system. Finally, although we currently have only a partial understanding of the mechanisms of MHC class II presentation in zebrafish, we can look to the cells that express MHC class II to inform us of their function and for a more complete understanding of the zebrafish adaptive immune system.

3. Zebrafish macrophages and DCs

3.1. Identification of macrophages

Zebrafish macrophages were first observed as phagocytic amoeboid cells on the yolk ball of developing embryos (Herbomel et al., 1999). They were initially distinguished from other cells types based on their morphology and differential expression of genetic markers in fixed tissues. For example, embryonic and larval macrophages can be distinguished from neutrophils by *in situ* hybridization to *csf1r/fms* transcript, which encodes MCSFR, a receptor that highly expressed in zebrafish and mammalian macrophages (Parichy et al., 2000). Additionally, macrophages produce low levels of myeloid peroxidase, a myeloid-specific peroxidase that can be detected histologically and is highly expressed in neutrophils (Lieschke et al., 2001).

Transgenic tools have been instrumental in the characterization of zebrafish leukocytes due to the lack of antibodies available for use in phenotyping zebrafish cell lineages. As they permit the live visualization of cells without physical disruption of tissues, transgenic lines are also useful for real-time imaging and fate-mapping experiments. Several transgenic lines are available to visualize macrophages during the early development of zebrafish (Table 1). Fluorescent reporters under the control of the *lyz* promoter faithfully mark lysozyme C-expressing cells, which include macrophages and granulocytes (Hall et al., 2007). As Pu.1 (Spi1) is a known regulator of myeloid and lymphoid lineages (Klemsz et al., 1990; Scott et al., 1994), an eGFP reporter for *pu.1* expression (*pu.1:eGFP*) accordingly marks lymphoid and myeloid fractions, including macrophages in zebrafish (Hsu et al., 2004). Finally, by discriminating between different levels of myeloperoxidase expression in *mpx:eGFP* fish, macrophages (GFP^{lo}) can be distinguished from neutrophils (GFP^{hi}) at early larval stages (Mathias et al., 2009). However, none of these reporters is specific to macrophages.

A number of genes are enriched in zebrafish macrophages, including *mpeg1*, whose expression is highly specific to macrophages in mammals. The *mpeg1*-promoter driven eGFP transgene preferentially marks macrophages in comparison to neutrophils in embryos and larvae up to 7 dpf, but its expression is not restricted to the macrophage lineage in adults (Ellett et al., 2011). It is also not clear if *mpeg1* is expressed in zebrafish DCs.

3.2. Identification of DCs

DCs were originally distinguished in mammals as cells with a distinct stellate morphology and later functionally defined as the most potent initiators of the adaptive immune response (Steinman and Cohn, 1973; Nussenzweig and Steinman, 1980). Upon encounter with antigen, DCs migrate to the T cell areas of local lymph nodes and other lymphatic tissues. A hallmark feature of DCs that distinguishes them from other MPs is the interaction of DCs with T cells in these organized lymphatic areas, which results in highly efficient priming of T cells.

Many different types of DCs have been identified in mammals based on divergent functional characteristics. The "classical" or "conventional" DCs have been historically subdivided into two subsets: CD8$^-$ (CD11b$^+$) DCs, which engage in conventional antigen presentation of external antigen upon MHC class II molecules and internal antigen upon MHC class I receptors, and CD8+ DCs (and related CD103$^+$ DCs in non-lymphoid tissues), which are thought to specialize in cross-presentation, or the presentation of processed external antigen on MHC class I molecules rather than MHC class II molecules (Hildner et al., 2008; Edelson et al., 2010). Plasmacytoid DCs (PDCs), a lineage of non-classical DCs, are specialized in the secretion of type I interferon and are important in the defense against acute and chronic viral infections (Reizis et al., 2011; Cervantes-Barragan et al., 2012).

Cells that morphologically resemble classical DCs have been identified in several teleost species, including rainbow trout (Bassity and Clark, 2012), medaka (Aghaallaei et al., 2010), and Atlantic salmon (Haugland et al., 2012). Zebrafish cells with dendritic morphology have been isolated from kidney marrow, a hematopoietic site akin to the mammalian bone marrow, based on their affinity for peanut agglutinin (PNA) (Lugo-Villarino et al., 2010). PNA^{hi} DC-like cells display similar characteristics to mammalian DCs as revealed by multiple cellular stains (Fig. 1). Differential PNA staining was also examined using an eGFP reporter for myeloperoxidase expression (*mpx:eGFP*). PNA^{hi} GFP-negative myeloid cells isolated from adult kidneys revealed enriched expression of DC-related genes, including *il12p40*, *csf1r*, and *iclp1* (encoding the invariant chain of the MHC class II complex). Furthermore, DC-like cells were shown to efficiently phagocytose labeled bacteria and exhibited

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010

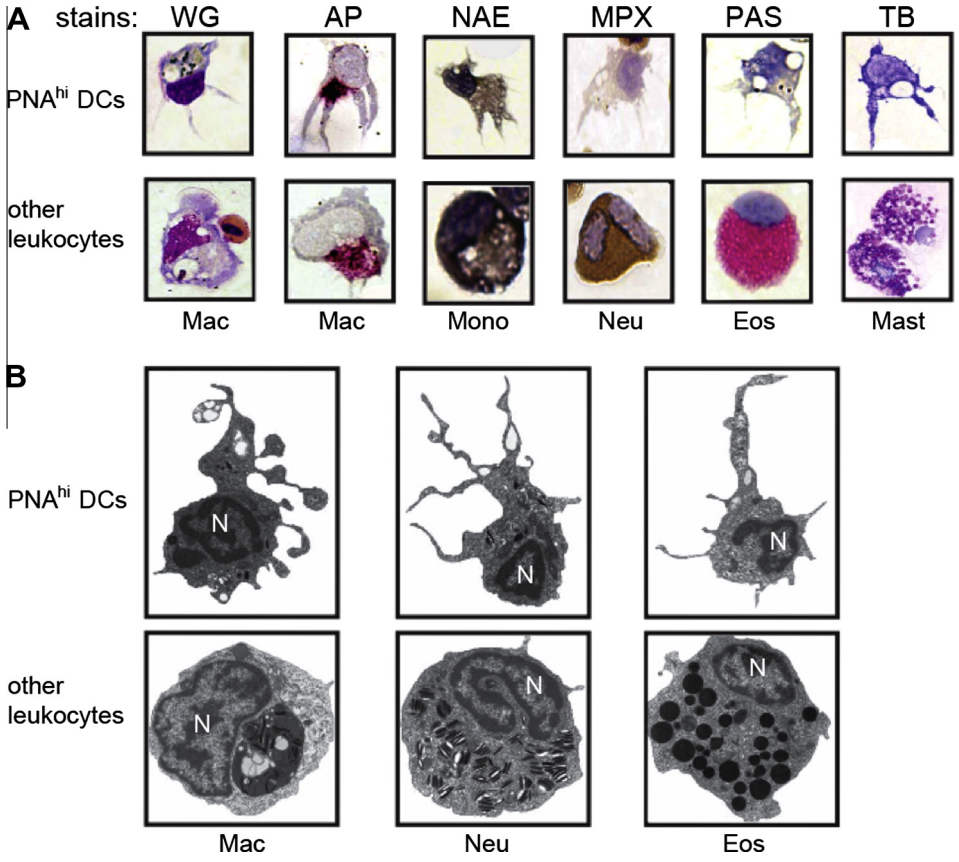

Fig. 1. Morphological characterization of zebrafish DCs. PNA^{hi} DC-like cells, macrophages (mac), monocytes (mono), neutrophils (neu), eosinophils (eos), and mast cells (mast) were isolated from zebrafish kidney marrow. Images originally published in Lugo-Villarino et al. (2010). (A) (Upper) Large dendrites are apparent on DC-like cells by Wright–Giemsa (WG) staining. DC-like cells contain small, acid phosphate (AP)-positive granules, seen as a magenta precipitate. DC-like cells also stain weakly for α-naphthyl acetate esterase (NAE), and are not stained by myeloperoxidase (MPX), periodic acid-Schiff reagent (PAS), and toluidine blue (TB). (Lower) Characteristic black precipitate is visible in the cytoplasm of monocytes by AP staining. Neutrophils, eosinophils, and mast cells all stain strongly for MPX (brown precipitate), PAS (red precipitate), and TB (purple precipitate). (B) Ultrastructure of (Upper) PNA^{hi} myelomonocytes and (Lower) other leukocytes by TEM. Nucleus (N).

antigen-specific T cell priming capacity *in vitro* (Lugo-Villarino et al., 2010).

Currently there are no transgenic tools available for the specific identification of DCs in zebrafish. However, a transgenic line that uses the regulatory region of the MHC class II *dab* gene (*mhc2dab*) to drive fluorescent reporter expression has been helpful in the characterization of multiple zebrafish APCs (Wittamer et al., 2011). When used in concert with a CD45 reporter line that distinguishes hematopoietic cells (*cd45:DsRed*), double positive (CD45$^+$; MHC2dab$^+$) myeloid cells were shown to contain a mixture of cells with either macrophage-like or DC-like morphologies and enriched expression of MP-related genes (Wittamer et al., 2011). Importantly, although the *cd45* transcript is detected in zebrafish B cells, the promoter/enhancer sequences used in the *cd45:DsRed* transgene do not activate marker transcription in zebrafish B cells. Therefore, use of this double transgenic line effectively distinguishes myeloid APCs from all other lineages. Unlike other APC-labeling transgenic lines, specific *mhc2dab:eGFP* expression within hematopoietic lineages is maintained throughout adulthood. This has enabled the characterization of zebrafish APCs from a variety of adult tissues, including the kidney marrow, spleen, skin, and gut ((Wittamer et al., 2011) and Fig. 2A). Morphological examination of the myeloid APCs in these tissues has revealed a tissue-specific distribution of DC-like cells. In particular, DC-like cells were found to be most abundant in the skin. Studies to further characterize zebrafish DCs and identify potential subsets of DCs have been limited by the lack of specific markers for their prospective identification. Additional tools are needed to probe the *in vivo* functions of DCs in zebrafish, including their activity during infections and in other immune-mediated processes such as tolerance, autoimmunity, and tumor immunity.

3.3. Development of the mononuclear phagocyte system

Macrophages and DCs belong to a larger class of mononuclear phagocytes (MPs), which includes monocytes, macrophages and DCs. Monocytes are circulating blood cells that produce inflammatory cytokines and differentiate into macrophages or DCs upon activation. They also mediate homeostatic processes in the steady-state. Although they are related to other MPs, monocytes do not express high levels of MHC class II (Van Voorhis et al., 1983), and are not considered to be major antigen-presenting cells. Zebrafish monocytes have not been formally characterized, and it is currently unknown if they play similar roles in homeostatic and immune processes or serve as precursors of MPs.

Our understanding of the pathways and precursors involved in the development of monocytes, macrophages, and DCs has undergone major revisions since the MP system was first described. It was long thought that monocytes serve as a major precursor to both DCs and macrophages. Although during infection, the activity of classical DCs is augmented with monocyte-derived inflammatory DCs, including TNF- and inducible nitric oxide synthase (iNOS)-producing DCs (Tip-DCs), DCs are not usually derived from monocytes under steady-state conditions (Naik et al., 2006). In the

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010

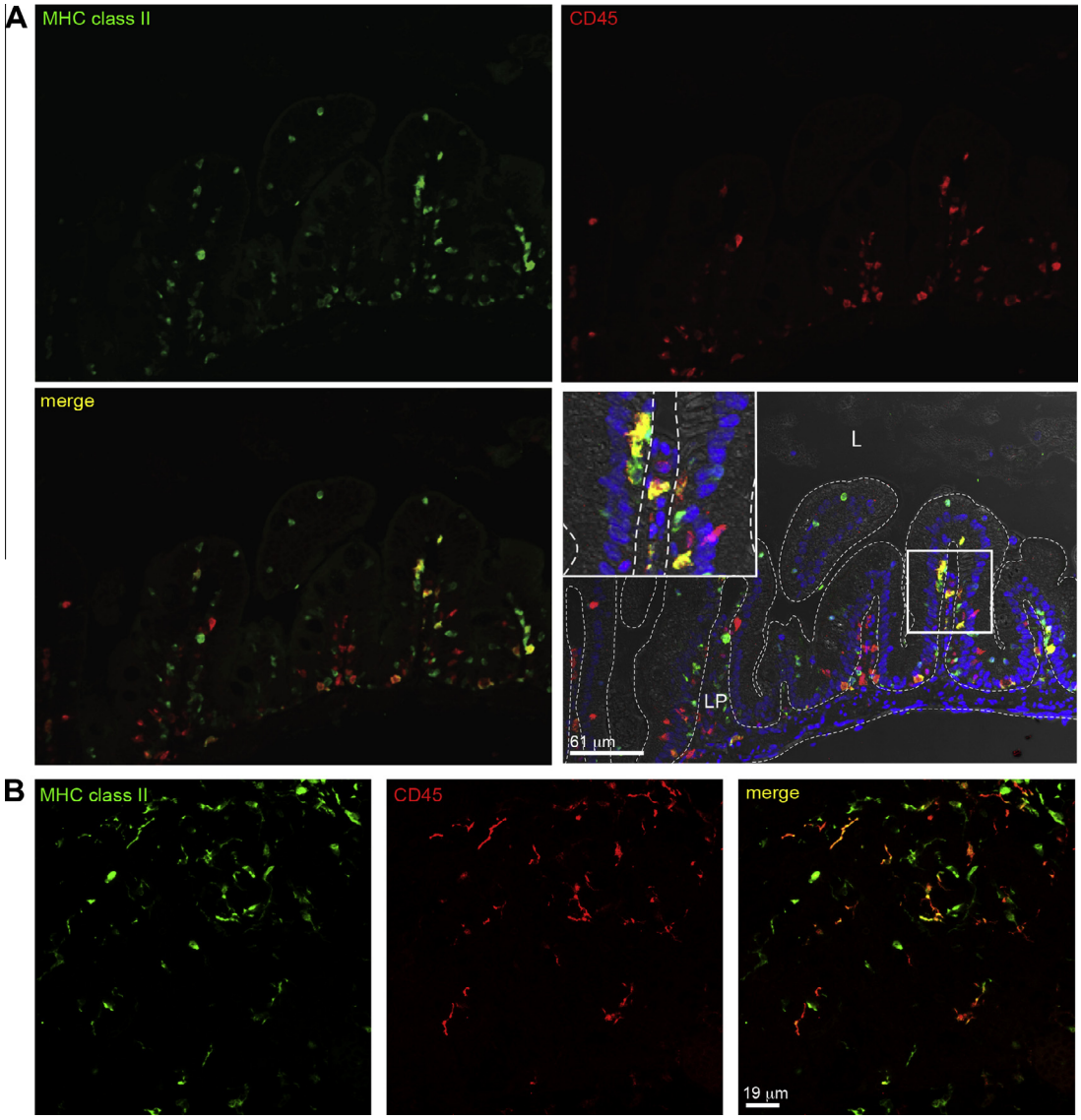

Fig. 2. Expression of the *mhc2dab:eGFP* (MHC class II) and *cd45:DsRed* (CD45) transgenes in the adult zebrafish. Immunohistochemistry staining of paraffin sections through the adult zebrafish (A) intestine and (B) brain in double transgenic *mhc2dab*:eGFP; *cd45:DsRed* animals. Sections were stained with anti-GFP (green) and anti-DsRed (red) antibodies. Nuclei are stained with DAPI (blue). Lamina propria (LP); lumen (L). $DsRed^+$ cells are expected to be T cells and/or neutrophils; $eGFP^+$ cells are expected to be B cells; $eGFP^+dsRed^+$ cells (yellow) are expected to be macrophages and/or dendritic cells. Sections were imaged by confocal microscopy and scale bars are shown.

absence of infection, murine DCs develop from preDCs in the spleen and other organs (Liu et al., 2009). There is currently very little known about zebrafish DC development, in part due to the lack of tools for their identification. It will be interesting to determine if zebrafish DCs arise through similar pathways as mammalian DCs under steady-state and inflammatory conditions and if zebrafish possess analogous differentiated subsets of DCs in lymphoid and non-lymphoid tissues.

It was also originally thought that all MPs differentiated from an HSC-derived common macrophage and DC progenitor (MDP). However, recent evidence suggests that several MP populations may originate from embryonic precursors and are maintained in adulthood without contribution from HSCs (Ginhoux et al., 2010; Schulz et al., 2012). Due to their early optical transparency, extra-utero growth, and well-characterized embryonic development, studies in zebrafish may help to resolve many unanswered questions about the contribution of embryonic precursors to adult MP lineages and the ontogeny of the MP system.

Macrophages are the earliest known immune cell type to arise during piscine and mammalian development. Primitive, or yolk sac macrophages, first appear in the yolk sac of zebrafish embryos within 24 hpf (Herbomel et al., 1999) and by E8 (Bertrand et al., 2005) in murine embryos. These cells have been observed to engulf apoptotic erythroblasts, eliminate pathogens during bacterial challenge, and migrate to sites of infection in the developing zebrafish embryo, suggesting that primitive macrophages participate in both homeostatic and immune processes (Herbomel et al., 1999).

Primitive macrophages have been implicated as precursors of microglia, a unique APC that resides in the brain. Though microglia are functionally and morphologically aligned with the macrophage lineage, fate-mapping experiments in mice have demonstrated that microglia arise from precursors that seed the brain before the development of the blood–brain barrier (Ginhoux et al., 2010). Concordantly, zebrafish yolk sac macrophages have been observed to colonize the brain and differentiate into cells that resemble microglia by 60 hpf (Herbomel et al., 2001). We have

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010

observed microglia-like cells in the brains of adult zebrafish using our *cd45:DsRed* and *mhc2dab:eGFP* double transgenic reporter line (Fig. 2B). It will be interesting to determine if the origin of these cells can also be traced to yolk sac precursors, which would suggest an evolutionary conservation of microglia origin.

Zebrafish primitive macrophages have also been observed to migrate to other peripheral tissues, including the epidermis at 24–48 hpf (Herbomel et al., 2001). This observation corresponds to findings that primitive macrophages may give rise to an epidermal APC in the mouse. Langerhans cells (LCs), a specific DC-like cell found in the epidermis, are thought to first develop from embryonic macrophages, but are gradually replaced by fetal liver monocytes (Hoeffel et al., 2012). Additionally, recent evidence suggests that a number of peripheral tissues are at least transiently colonized by yolk sac-derived precursors (Schulz et al., 2012).

Subsequent fate-mapping studies in mice have demonstrated that several tissue-resident macrophages are maintained into adulthood without contribution from circulating monocytes (Yona et al., 2013; Hashimoto et al., 2013). These studies indicate that microglia are not the only MP population that is seeded by embryonic or prenatal precursors and maintained independently of HSCs. However, it remains unclear how long these lineages persist in adults, and, except in the case of microglia, which presumably must be seeded before the blood–brain barrier is formed, the significance of this early colonization and distinct origin is not certain.

Further insights into the origin of MPs may be gained by examining the signaling pathways required for their development. The MCSF receptor (MCSFR) is associated with the development of macrophages, and MCSFR-deficient mice lack macrophages (Dai et al., 2002). However, circulating monocytes are present in MCSFR-deficient mice, suggesting either that MCSFR signaling is required for the monocyte to macrophage transition, or that monocytes do not necessarily serve as a major macrophage precursor (Ginhoux et al., 2010). Interestingly, primitive macrophages were observed to develop and function normally, but failed to migrate out of the yolk sac in zebrafish *panther* mutants, which lack functional MCSFR (Herbomel et al., 2001). Both yolk sac macrophages and microglia failed to form in MCSFR-deficient mice, indicating that at least microglial dependence on MCSFR is conserved across species (Ginhoux et al., 2010).

Recent studies in mice have challenged the notion that macrophages originate from adult monocytes, proposing instead that many tissue-resident macrophages arise from precursors that are seeded prior to birth and maintained without contribution from adult HSCs. With a well-developed arsenal of fate-mapping tools and their amenability to live imaging, zebrafish may prove to be an exceptional model to gain further insights into the origin of peripheral tissue macrophages.

4. Zebrafish B cells

4.1. B cell identification and characterization

B lymphocytes express MHC class II, can efficiently internalize antigen through B cell receptors (BCRs), and are able to locate and interact with reactive $CD4^+$ T cells in secondary lymphoid organs. BCRs have the potential to recognize a vast array of foreign antigens, and upon BCR cross-linking, immature B cells can differentiate into plasma and memory cells, providing immediate pathogen-specific antibodies and long-lasting immunological memory.

Mammals express five immunoglobulin isotypes (IgM, IgD, IgG, IgE, and IgA), each of which has a distinct role determined by its constant region. For example, whereas IgG is found in high titers in serum, IgA is the principal antibody that mediates mucosal immunity. Three immunoglobulin heavy chain (IgH) isotypes have been identified in zebrafish: μ, δ, and ζ, corresponding to IgM, IgD, and IgZ, respectively. As in mammals, IgM and IgD can be expressed on the same B cell in channel catfish (Edholm et al., 2010), but it is unclear if zebrafish B cells similarly co-express these two immunoglobulins.

The IgZ (IgT in trout) isotype is unique to teleosts. The ζ locus, with its own D and joining (J) exons, is embedded between the variable (V) and diversity (D) exons used by the μ and δ isotypes (Danilova et al., 2005). Hence, the expression of IgM/D may exclude IgZ expression, as the ζ gene segment is excised from the genome during IgM/IgD VDJ recombination. There are no additional isotypes downstream of the δ locus or switch regions present in the zebrafish heavy chain locus, both of which are present and required for class switch recombination (CSR) in amphibians and higher vertebrates (reviewed in Stavnezer and Amemiya (2004)). Despite the absence of CSR in zebrafish, they and other teleosts express an orthologue of activation-induced deaminase (AID) that is able to catalyze CSR when expressed ectopically in murine B cells (Barreto et al., 2005; Conticello et al., 2005).

It is suggested that the various IgM species observed in teleosts (monomers, dimers, trimers, tetramers, with varying degrees of cross-linking) and post-translational modifications may result in different IgM effector functions, analogous to the multiple antibody isotypes present in mammals (Costa et al., 2012). Hence, zebrafish may employ alternative processes to CSR in order to carry out specific effector functions. Finally, IgT seems to be specialized for mucosal immunity in trout (Zhang et al., 2010). Thus, immunoglobulin specification appears to have arisen in at least one teleost species independently of CSR. Whether or not zebrafish IgZ has a role in mucosal immunity is currently unknown.

4.2. B cell development

Upon specification, mammalian hematopoietic stem cells (HSCs) populate the fetal liver, an intermediate site of hematopoiesis and B cell development. As the organism matures, the site of hematopoiesis and B cell development shifts to the bone marrow, and remains there throughout adulthood. In the zebrafish, newly specified HSCs seed the caudal hematopoietic tissue, a short-lived site of hematopoiesis analogous to the mammalian fetal liver. HSCs subsequently seed the kidney marrow, which is the site of hematopoiesis and B lymphopoiesis in the adult teleost.

Several studies have indicated that zebrafish B cells develop around 2–3 wpf. Zebrafish Ig light chain (*IgLC*) transcript levels are low, but can be detected as early as 3 dpf by RT-PCR analysis; levels dramatically increase by 3 wpf (Lam et al., 2004). *Rag1* and *IgLC* are first detected in the zebrafish kidney by 2 and 3 wpf, respectively, as assessed by *in situ* hybridization to tissue sections (Lam et al., 2004). Also, although rearranged μ genes can be detected by PCR as early as 4 dpf, membrane-bound and secreted IgM are not detected by RT-PCR until 7 and 13 dpf, respectively (Danilova and Steiner, 2002). Ig protein is not detected until 4 wpf (Lam et al., 2004). Additionally, zebrafish did not mount a measurable primary humoral response to formalin-killed *Aeromonas hydrophila* until 7 wpf, and a modest secondary response was not observed until 10 wpf (Lam et al., 2004). Hence, zebrafish B cells begin to undergo VDJ recombination at 4 dpf, are detected in the kidney by 3 wpf, and are capable of mounting primary responses by 7 wpf and secondary responses by 10 wpf.

Surprisingly, Danilova and Steiner identified the pancreas as an early site of zebrafish B cell lymphopoiesis, visualizing *rag1* and *igμ* transcript in the pancreas at 4 and 10 dpf, respectively, by whole mount *in situ* hybridization (Danilova and Steiner, 2002). However, we were unable to observe pancreatic B cells between 4 and 10 dpf using a transgenic fish we generated that labels IgM^+ B cells (*igm:eGFP*) (Page et al., 2013). We did, however, observe $Rag2^+IgM^+$

cells in the kidney at 3 wpf. Clusters of $Rag2^+IgM^+$ cells were also found near the posterior cardinal vein (Page et al., 2013). Our data suggest that there may be either shifting sites of B cell emergence, or multiple sites throughout the lifespan of the animal. The former explanation is analogous to mammalian B cell development, which begins in the fetal liver and then shifts to the bone marrow.

A number of important transcription factors that are involved in the development and egress of immature mammalian B cells appear to be well conserved in zebrafish. The zinc-finger transcription factor Ikaros is expressed in HSCs and required for B and T cell development in mammals (Wang et al., 1996; Georgopoulos et al., 1994). An Ikaros homolog has been identified in zebrafish, and, as in mammals, it is expressed at the site of HSC emergence and the thymus of developing zebrafish (Willett et al., 2001). Functional studies have revealed a conserved role for Ikaros in zebrafish and mammals. A loss-of-function Ikaros mutant in mice results in the absence of fetal B and T cells, and postnatal B cells (Wang et al., 1996). A zebrafish mutant expressing a similarly truncated Ikaros protein was generated and was also lacking in both larval IgZ^+ B and T cells, and IgZ^+ B cells in the adult, indicating that a conserved program for lymphocyte development exists among mammals and zebrafish (Schorpp et al., 2006). Notably, IgM^+ B cells were less affected by the Ikaros truncation compared to IgZ^+ B cells (Schorpp et al., 2006). This differential requirement for Ikaros suggests that IgM^+ and IgZ^+ B cell lineages may be divergently regulated.

The transcription factor Pax5 is involved in fate determination during early B cell development. Blimp1 and Xbp1 are transcription factors active in terminally differentiating B cells. Homologues of all three genes have been isolated in zebrafish [reviewed in Zwollo (2011)]. Zebrafish Xbp1 appears to be functionally similar to mammalian Xbp1, as zebrafish Xbp1 was activated and upregulated genes involved in ER stress in response to tunicamycin (an N-glycosylation inhibitor and inducer of ER-stress) in a zebrafish cell line (Hu et al., 2007).

E2A is a helix-loop-helix transcription factor, a member of the E-protein family (which also includes HEB and E2-2), and is involved in mammalian B cell development and CSR. E2A has not been identified in zebrafish, but an E2A homolog has been identified in catfish (Hikima et al., 2005). However, alternative E proteins may compensate for E2A function in teleost B cells. For example, the HEB homolog CFEB1 induces greater activation of a reporter containing an IgH enhancer compared to E2A in a catfish B cell line (Hikima et al., 2005). Overall, the function of transcription factors at multiple stages of B cell development appears to be conserved between zebrafish and mammals. Further characterization of transcription factors specific to zebrafish B cells may prove to be useful in generating novel transgenic lines that label the various developmental stages of B cells.

4.3. Generation of diversity

The early B cell repertoire is created by combinatorial VDJ rearrangement and junctional diversity. The repertoire further diversifies during an immune response by clonal expansion and somatic hypermutation (SHM). A methodical study examined the VDJμ repertoire in zebrafish (Jiang et al., 2011). Similar to mammals, young (2 wpf) zebrafish have limited repertoires (200 out of a possible 975 VDJμ combinations). Additionally, an identical core group of VDJ combinations are used between fish, suggesting that the initial repertoire is, to some extent, pre-determined (Jiang et al., 2011). By 3 wpf, about 700 combinations are observed per fish. As the fish aged, the presence of mutated VDJ segments increased, indicative of somatic mutation. Examining the evolution of the immune repertoire through the developmental stages of an organism is a daunting task in mice, as there the combinatorial potential of the heavy chain is over 15,000. However the combinatorial potential for the VDJ region of the zebrafish μ gene is only 975. Thus, the zebrafish may be an attractive model to examine the development of the antibody repertoire.

Mammalian B cells expand, undergo SHM, affinity maturation, and CSR in the germinal centers of secondary lymphoid organs. The germinal center reaction gives rise to high affinity antibody-secreting plasma cells and long-lived memory B cells (reviewed in MacLennan (1994)). Zebrafish and other teleosts lack lymph nodes and fail to form robust germinal centers. As mammalian plasma and memory B cells primarily develop in germinal centers, it is of interest to understand how the teleost is able to promote humoral responses and the extent of their secondary responses.

Teleost B cells undergo many of the same processes that occur in mammalian germinal centers even though they do not form robust germinal centers. Rainbow trout IgM+ and IgT+ B cells undergo splenic clonal expansion in response to systemic viral infection (Castro et al., 2013). Although teleost B cells do not class switch, SHM of immunoglobulin genes is evident in zebrafish (Marianes and Zimmerman, 2011; Jiang et al., 2011) and channel catfish (Yang et al., 2006). Additionally, affinity maturation has been measured in rainbow trout, although antibody affinities increase marginally compared to the logarithmic increases that are measured in mammals (Cain et al., 2002; Kaattari et al., 2002; Ye et al., 2011).

Notably, the generation of germinal centers is not an absolute requirement for affinity maturation in mice. Lymphotoxin-α-deficient ($LT\alpha^{-/-}$) mice do not produce germinal centers and lack organized follicular DC (FDC) clusters. However, $LT\alpha^{-/-}$ mice immunized with a high antigen dose undergo CSR, and produce high affinity antibodies to the same extent as immunized WT mice (Matsumoto et al., 1996). It is not clear if dispersed FDCs, or another cell type, mediate affinity maturation in these mice. Finally, it has been suggested that clusters of splenic AID-expressing cells found in association with melano-macrophages (a cell type capable of retaining whole antigen on its surface) may represent "primordial germinal centers" in channel catfish (Saunders et al., 2010). The *igm:eGFP* (Page et al., 2013) and *blimp1:eGFP* (Elworthy et al., 2008) zebrafish can be used to begin to elucidate the requirements, locations, and cells involved in the generation of zebrafish memory and plasma B cells (Page et al., 2013).

4.4. Zebrafish B cell phagocytosis

Unexpectedly, rainbow trout and catfish IgM^+ B cells were shown to be able to phagocytose latex beads and bacteria (Li et al., 2006). Moreover, the B cells could also kill internalized bacteria. Additional studies revealed that catfish, Atlantic cod, and Atlantic salmon IgM^+ B cells could also phagocytose particles *in vitro* (Overland et al., 2010; Li et al., 2006). Zebrafish IgM^+ B cells, however, had little phagocytic capacity for either beads or bacteria (Page et al., 2013), suggesting teleost B cells are not equally phagocytic.

Following observations of the phagocytic capacity of certain teleost B cells, it was recently demonstrated that murine B-1 cells from the peritoneal cavity also had phagocytic and bactericidal properties (Parra et al., 2012). In addition, peritoneal cavity B-1 cells were able to process and present ingested antigen to $CD4^+$ T cells (Parra et al., 2012). Unlike conventional B-2 cells, B-1 cells are innate immune cells that have limited, germline-encoded BCR repertoires, secrete "natural" IgM antibodies with broad specificity and do not directly participate in adaptive responses. Based on their shared phagocytic and bactericidal capacity, it has been proposed that teleost B cells more closely resemble mammalian B-1 cells than conventional B-2 cells, suggesting that phagocytic innate-like B cells may have emerged prior to B-2 cells (Sunyer, 2013). The recent finding of phagocytic mammalian B-1 cells highlights the importance of continuing efforts to better characterize

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010

the immune cells of lower vertebrates for a more complete understanding of the mammalian immune system.

5. The function of APCs in zebrafish

Little is known about the activity of zebrafish APCs during infection and other immune-mediated processes, including tolerance, autoimmunity and tumor immunity. In fact, the importance of T cell priming and adaptive immune-mediated protection has never been conclusively demonstrated in zebrafish. Due to their optical transparency and ease of genetic manipulation in the embryonic and larval stages, most studies on the function of APCs have focused on their role in innate immunity during early development. These studies have led to significant advancements, particularly in the understanding of mycobacterial infection. However, very few studies have examined the role of APCs in adult zebrafish. The development of transgenic tools that reliably label APCs in adults, including *mhc2dab:eGFP* and *igm:eGFP*, has aided in the characterization of APCs in adult organs. These tools can be used to further visualize immune cell interactions, including antigen presentation, and to test the role of adaptive immunity in zebrafish.

5.1. APCs in innate and adaptive immune responses

The activity of zebrafish APCs has been best characterized during early life, before pigment formation obstructs facile observation. However, as the adaptive immune system does not fully form until at least 2–3 weeks post fertilization, these studies have necessarily focused on the role of APCs in innate immune responses. Primitive macrophages have been observed to phagocytose apoptotic cells, clear bacteria, and migrate toward sites of bacterial injection (Herbomel et al., 1999). Larval macrophages and neutrophils migrated to the site of *Streptococcus iniae* infection and both cell types were observed to actively phagocytose bacteria (Harvie et al., 2013). Targeted depletion of myelo-erythroid cells, including neutrophils and macrophages, in *pu.1* morphants, led to increased susceptibility to *S. iniae* infection.

The role of macrophages during *Mycobacterium marinum* infection of zebrafish embyros and larvae has been extensively studied. Infection with *M. marinum*, a close relative of *Mycobacterium tuberculosis*, causes macrophages to aggregate into structures that closely resemble the hallmark granulomas that form in the lungs of tuberculosis patients (Pozos and Ramakrishnan, 2004). As this process has been poorly modeled in mice, zebrafish have become an invaluable tool for the study of granuloma formation and innate immune responses in tuberculosis.

Macrophages rapidly migrate to sites of mycobacteria injection (Clay et al., 2007). This migration was shown to be specific to the presence of bacteria, as injection of latex beads did not provoke a similar response. Although neutrophils are capable of uptaking bacteria, macrophages were shown to be the major cell involved in phagocytosing *M. marinum* during the initial stages of infection. Bacterial load was significantly expanded, but restricted to the site of infection in the absence of macrophages, indicting that macrophages initially inhibit *M. marinum* growth, but also inadvertently disseminate the pathogen to other tissues (Clay et al., 2007).

Activated immature B cells can differentiate into memory B cells during a primary immune response. Memory B cells mediate quicker and more robust secondary immune responses upon re-exposure to antigen, the hallmarks of mammalian adaptive immunity. In general, teleost secondary B cell responses appear to be slightly quicker and marginally more robust compared to primary responses (Lam et al., 2004; Arkoosh and Kaattari, 1991; Trump and Hildemann, 1970). The common carp may be an exception among teleosts with significantly more robust response to secondary immunizations (Rijkers et al., 1981; Lamers et al., 1985).

We found that zebrafish immunized with a soluble antigen do not exhibit a shorter lag phase or more robust secondary response as assessed by the number or percentage of IgM^+ B cells (Page et al., 2013). However, antibody titers were not measured in this study. Furthermore, the participation of IgZ^+ B cells in secondary responses was not evaluated.

Although it has been well demonstrated that teleost B cells proliferate and secrete pathogen-specific antibody (Zhang et al., 2010; Xu et al., 2013; Cain et al., 2002; Castro et al., 2013; Chinchilla et al., 2013), the importance of humoral immunity in controlling infections in zebrafish is unclear. For example, zebrafish B cells appear to have little to no role in the response against *Edwardsiella tarda* (Yang et al., 2013). Additionally, $Rag1^{-/-}$ zebrafish, which lack functional T and B cells, immunized with *Edwardsiella ictaluri* demonstrate greater survival against subsequent specific challenge compared to non-immunized fish (Petrie-Hanson et al., 2009; Hohn and Petrie-Hanson, 2012). Thus, zebrafish may be capable of mounting an acquired immune response in the absence of B and T cell activity. Overall, experimental settings where B cell and immunoglobulin responses are measured indicate that, in comparison to mammalian B cells, teleost B cells respond more slowly to initial challenge with antigen and undergo little to no affinity maturation, but can mount weak secondary responses (Arkoosh and Kaattari, 1991; Cain et al., 2002; Trump and Hildemann, 1970).

5.2. The activity of zebrafish APCs at environmental interfaces

5.2.1. APCs in intestinal immunity

The mammalian intestinal immune system is comprised of multiple tissue-specific and circulating immune cell types. There are two main antigen-sampling cells in the mammalian gut: M cells and lamina propria DCs. M cells are specialized cells that sit in the epithelial layer lining Peyer's patches (PPs) and isolated lymphoid follicles, and take up antigen from the intestinal lumen. Microbes and antigens that penetrate the epithelium through M cells are met by a host of organized immune cells in PPs and isolated lymphoid follicles. Both of these tissues drain to the mesenteric lymph node (MLN).

The lamina propria (LP) is a layer of tissue beneath the epithelial cells of the villi that contains several different types of immune cells, including B and T lymphocytes, macrophages, mast cells, and dendritic cells. All of these cells have also been observed in the intestines of teleosts (reviewed in Gomez et al. (2013)). Mammalian LP DCs can partially penetrate the epithelial layer to access antigens from the lumen of the gut (Niess et al., 2005). LP DCs then migrate to the MLN where they present captured antigen. DC-like cells have been isolated from zebrafish intestine, but it has not been determined if these cells similarly extend into the lumen (Wittamer et al., 2011). In contrast to mammals, where a single B cell isotype (IgA^+ B cells) normally predominates in the gut, both IgM^+ and IgT^+ B cells are found at similar levels in the trout intestine (Zhang et al., 2010). However, IgT^+ B cells are the major B cell type found in the trout intestine during infection, suggesting that IgT/IgZ^+ B cells, like IgA^+ B cells, may be specialized in mucosal immunity (Zhang et al., 2010). The distribution of intestinal IgM^+ versus IgZ^+ B cells under homeostatic and inflammatory conditions has yet to be determined in zebrafish.

Outside of the LP, the gut-associated lymphoid tissue (GALT) of teleosts is poorly defined. Teleosts apparently lack PPs and MLN, and although M cell-like antigen-sampling cells have been observed in salmonids, M cells have not been identified in zebrafish (Fuglem et al., 2010). Therefore, despite significant similarities in the resident populations of immune cells in the gut, it is unclear

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010

how antigens are sampled from the intestinal lumen and where presentation of these antigens occurs in teleosts. Greater understanding of the unique and conserved features of the zebrafish intestine will further validate its use as a model of host-commensal interactions and intestinal immunity.

5.2.2. APCs in the skin

Teleost skin differs from mammalian skin in that it lacks an outer layer of keratinized epithelial cells and instead possesses a mucus layer, similar to the intestine and gills (Gomez et al., 2013). Recently, the teleost skin has been proposed as a mucosal organ due to the prevalence of IgT^+ B cells that accumulate in the epidermis in response to infection (Xu et al., 2013). IgT^+ B cells are normally found at low levels in trout skin but increased along with skin mucus IgT levels during infection with *Ichthyophthirius multifiliis*. In contrast, IgM^+ B cell numbers and mucosal IgM levels were unaffected by infection.

In addition to B cells, DC-like cells and macrophages have been observed in zebrafish skin (Wittamer et al., 2011). In mammals, several myeloid APCs reside in the skin: LCs, which are the only MPs found the epidermis, conventional and monocyte-derived dermal DC and dermal macrophages (Tamoutounour et al., 2013). LC and dermal DCs migrate to skin-draining lymph nodes (Henri et al., 2010). However, like other lymph nodes, cutaneous lymph nodes have not been identified in zebrafish. Additionally, the epidermal and dermal resident MP populations of zebrafish have not been thoroughly investigated.

6. Perspectives on APCs in zebrafish

In comparison to mammals where the importance of adaptive immunity has been clearly demonstrated, there is very little evidence showing similar dependence in zebrafish. In fact, Ikaros mutant fish, which lack most B and T cells do not have impaired survival rates (Schorpp et al., 2006). Furthermore, Rag1 mutant fish, which completely lack B and T lymphocytes, are remarkably resistant to secondary infection and are suggested to achieve specific protective immunity through innate mechanisms (Petrie-Hanson et al., 2009; Hohn and Petrie-Hanson, 2012). This contrasts with the apparent susceptibility of zebrafish to infection in the absence of key innate immune components. MyD88 mutant fish succumb rapidly to infections (van der Vaart et al., 2013), while MyD88-deficient humans are relatively resistant to many pathogens (von Bernuth et al., 2008). This difference could point to variations in the requirement of specific adaptor proteins between species or may indicate that zebrafish rely more heavily on innate mechanisms of immunity compared to mammals.

Notably, the faster and more robust immune response that defines adaptive immunity in higher vertebrates has not been consistently observed in zebrafish. Furthermore, there is currently little evidence that affinity maturation plays a major role in fine-tuning secondary responses to infection. This observation is consistent with apparent lack of lymph nodes, Peyer's patches, germinal centers, or other lymphoid tissue organization in zebrafish. Although certain organized lymphoid tissues may exist in other teleosts and even in lower vertebrates, it is possible that zebrafish possess a unique iteration of the immune system that includes the cellular components of the adaptive immune system, but lacks the structures that facilitate antigen presentation and other fine-tuned interactions between cells.

The potential use of zebrafish in studies of adaptive immunity is critically dependent on further characterization of the similarities and differences between mammalian and zebrafish APCs. Finally, while studies in mammals have revealed an ever-expanding range of APC subsets, simpler model organisms provide us with the opportunity to discover the essential unifying features of these cell types.

References

Aghaallaei, N., Bajoghli, B., Schwarz, H., Schorpp, M., Boehm, T., 2010. Characterization of mononuclear phagocytic cells in medaka fish transgenic for a cxcr3a:gfp reporter. Proc. Natl. Acad. Sci. USA 107, 18079–18084.

Arkoosh, M.R., Kaattari, S.L., 1991. Development of immunological memory in rainbow trout (Oncorhynchus mykiss). I. An immunochemical and cellular analysis of the B cell response. Dev. Comp. Immunol. 15, 279–293.

Barreto, V.M., Pan-Hammarstrom, Q., Zhao, Y., Hammarstrom, L., Misulovin, Z., Nussenzweig, M.C., 2005. AID from bony fish catalyzes class switch recombination. J. Exp. Med. 202, 733–738.

Bassity, E., Clark, T.G., 2012. Functional identification of dendritic cells in the teleost model, rainbow trout (Oncorhynchus mykiss). PLoS ONE 7, e33196.

Bertrand, J.Y., Jalil, A., Klaine, M., Jung, S., Cumano, A., Godin, I., 2005. Three pathways to mature macrophages in the early mouse yolk sac. Blood 106, 3004–3011.

Bertrand, J.Y., Kim, A.D., Teng, S., Traver, D., 2008. CD41+ cmyb+ precursors colonize the zebrafish pronephros by a novel migration route to initiate adult hematopoiesis. Development 135, 1853–1862.

Blum, J.S., Wearsch, P.A., Cresswell, P., 2013. Pathways of antigen processing. Annu. Rev. Immunol. 31, 443–473.

Cain, K.D., Jones, D.R., Raison, R.L., 2002. Antibody-antigen kinetics following immunization of rainbow trout (Oncorhynchus mykiss) with a T-cell dependent antigen. Dev. Comp. Immunol. 26, 181–190.

Castro, R., Jouneau, L., Pham, H.P., Bouchez, O., Giudicelli, V., Lefranc, M.P., Quillet, E., Benmansour, A., Cazals, F., Six, A., Fillatreau, S., Sunyer, O., Boudinot, P., 2013. Teleost fish mount complex clonal IgM and IgT responses in spleen upon systemic viral infection. PLoS Pathog. 9, e1003098.

Cervantes-Barragan, L., Lewis, K.L., Firner, S., Thiel, V., Hugues, S., Reith, W., Ludewig, B., Reizis, B., 2012. Plasmacytoid dendritic cells control T-cell response to chronic viral infection. Proc. Natl. Acad. Sci. USA 109, 3012–3017.

Chinchilla, B., Gomez-Casado, E., Encinas, P., Falco, A., Estepa, A., Coll, J., 2013. In vitro neutralization of viral hemorrhagic septicemia virus by plasma from immunized zebrafish. Zebrafish 10, 43–51.

Clay, H., Davis, J.M., Beery, D., Huttenlocher, A., Lyons, S.E., Ramakrishnan, L., 2007. Dichotomous role of the macrophage in early Mycobacterium marinum infection of the zebrafish. Cell Host Microbe 2, 29–39.

Conticello, S.G., Thomas, C.J., Petersen-Mahrt, S.K., Neuberger, M.S., 2005. Evolution of the AID/APOBEC family of polynucleotide (deoxy)cytidine deaminases. Mol. Biol. Evol. 22, 367–377.

Costa, G., Danz, H., Kataria, P., Bromage, E., 2012. A holistic view of the dynamisms of teleost IgM: a case study of Streptococcus iniae vaccinated rainbow trout (Oncorhynchus mykiss). Dev. Comp. Immunol. 36, 298–305.

Dai, X.M., Ryan, G.R., Hapel, A.J., Dominguez, M.G., Russell, R.G., Kapp, S., Sylvestre, V., Stanley, E.R., 2002. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. Blood 99, 111–120.

Danilova, N., Bussmann, J., Jekosch, K., Steiner, L.A., 2005. The immunoglobulin heavy-chain locus in zebrafish: identification and expression of a previously unknown isotype, immunoglobulin Z. Nat. Immunol. 6, 295–302.

Danilova, N., Steiner, L.A., 2002. B cells develop in the zebrafish pancreas. Proc. Natl. Acad. Sci. USA 99, 13711–13716.

Dijkstra, J.M., Grimholt, U., Leong, J., Koop, B.F., Hashimoto, K., 2013. Comprehensive analysis of MHC class II genes in teleost fish genomes reveals dispensability of the peptide-loading DM system in a large part of vertebrates. BMC Evol. Biol. 13, 260.

Edelson, B.T., Kc, W., Juang, R., Kohyama, M., Benoit, L.A., Klekotka, P.A., Moon, C., Albring, J.C., Ise, W., Michael, D.G., Bhattacharya, D., Stappenbeck, T.S., Holtzman, M.J., Sung, S.S., Murphy, T.L., Hildner, K., Murphy, K.M., 2010. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. J. Exp. Med. 207, 823–836.

Edholm, E.S., Bengten, E., Stafford, J.L., Sahoo, M., Taylor, E.B., Miller, N.W., Wilson, M., 2010. Identification of two IgD+ B cell populations in channel catfish, Ictalurus punctatus. J. Immunol. 185, 4082–4094.

Ellett, F., Pase, L., Hayman, J.W., Andrianopoulos, A., Lieschke, G.J., 2011. Mpeg1 promoter transgenes direct macrophage-lineage expression in zebrafish. Blood 117, e49–e56.

Elworthy, S., Hargrave, M., Knight, R., Mebus, K., Ingham, P.W., 2008. Expression of multiple slow myosin heavy chain genes reveals a diversity of zebrafish slow twitch muscle fibres with differing requirements for Hedgehog and Prdm1 activity. Development 135, 2115–2126.

Fuglem, B., Jirillo, E., Bjerkas, I., Kiyono, H., Nochi, T., Yuki, Y., Raida, M., Fischer, U., Koppang, E.O., 2010. Antigen-sampling cells in the salmonid intestinal epithelium. Dev. Comp. Immunol. 34, 768–774.

Georgopoulos, K., Bigby, M., Wang, J.H., Molnar, A., Wu, P., Winandy, S., Sharpe, A., 1994. The Ikaros gene is required for the development of all lymphoid lineages. Cell 79, 143–156.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M.F., Conway, S.J., Ng, L.G., Stanley, E.R., Samokhvalov, I.M., Merad, M., 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330, 841–845.

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010

Gomez, D., Sunyer, J.O., Salinas, I., 2013. The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol. 35, 1729–1739.
Graser, R., Vincek, V., Takami, K., Klein, J., 1998. Analysis of zebrafish Mhc using BAC clones. Immunogenetics 47, 318–325.
Hall, C., Flores, M.V., Storm, T., Crosier, K., Crosier, P., 2007. The zebrafish lysozyme C promoter drives myeloid-specific expression in transgenic fish. BMC Dev. Biol. 7, 42.
Harvie, E.A., Green, J.M., Neely, M.N., Huttenlocher, A., 2013. Innate immune response to Streptococcus iniae infection in zebrafish larvae. Infect. Immun. 81, 110–121.
Hashimoto, D., Chow, A., Noizat, C., Teo, P., Beasley, M.B., Leboeuf, M., Becker, C.D., See, P., Price, J., Lucas, D., Greter, M., Mortha, A., Boyer, S.W., Forsberg, E.C., Tanaka, M., van Rooijen, N., Garcia-Sastre, A., Stanley, E.R., Ginhoux, F., Frenette, P.S., Merad, M., 2013. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 38, 792–804.
Haugland, G.T., Jordal, A.E., Wergeland, H.I., 2012. Characterization of small, mononuclear blood cells from salmon having high phagocytic capacity and ability to differentiate into dendritic like cells. PLoS ONE 7, e49260.
Henri, S., Poulin, L.F., Tamoutounour, S., Ardouin, L., Guilliams, M., de Bovis, B., Devilard, E., Viret, C., Azukizawa, H., Kissenpfennig, A., Malissen, B., 2010. CD207+ CD103+ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells. J. Exp. Med. 207, 189–206.
Herbomel, P., Thisse, B., Thisse, C., 1999. Ontogeny and behaviour of early macrophages in the zebrafish embryo. Development 126, 3735–3745.
Herbomel, P., Thisse, B., Thisse, C., 2001. Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. Dev. Biol. 238, 274–288.
Hikima, J., Middleton, D.L., Wilson, M.R., Miller, N.W., Clem, L.W., Warr, G.W., 2005. Regulation of immunoglobulin gene transcription in a teleost fish: identification, expression and functional properties of E2A in the channel catfish. Immunogenetics 57, 273–282.
Hildner, K., Edelson, B.T., Purtha, W.E., Diamond, M., Matsushita, H., Kohyama, M., Calderon, B., Schraml, B.U., Unanue, E.R., Diamond, M.S., Schreiber, R.D., Murphy, T.L., Murphy, K.M., 2008. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. Science 322, 1097–1100.
Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., Leboeuf, M., Low, D., Oller, G., Almeida, F., Choy, S.H., Grisotto, M., Renia, L., Conway, S.J., Stanley, E.R., Chan, J.K., Ng, L.G., Samokhvalov, I.M., Merad, M., Ginhoux, F., 2012. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. J. Exp. Med. 209, 1167–1181.
Hohn, C., Petrie-Hanson, L., 2012. Rag1−/− mutant zebrafish demonstrate specific protection following bacterial re-exposure. PLoS ONE 7, e44451.
Hsu, K., Traver, D., Kutok, J.L., Hagen, A., Liu, T.X., Paw, B.H., Rhodes, J., Berman, J.N., Zon, L.I., Kanki, J.P., Look, A.T., 2004. The pu.1 promoter drives myeloid gene expression in zebrafish. Blood 104, 1291–1297.
Hu, M.C., Gong, H.Y., Lin, G.H., Hu, S.Y., Chen, M.H., Huang, S.J., Liao, C.F., Wu, J.L., 2007. XBP-1, a key regulator of unfolded protein response, activates transcription of IGF1 and Akt phosphorylation in zebrafish embryonic cell line. Biochem. Biophys. Res. Commun. 359, 778–783.
Jiang, N., Weinstein, J.A., Penland, L., White 3rd, R.A., Fisher, D.S., Quake, S.R., 2011. Determinism and stochasticity during maturation of the zebrafish antibody repertoire. Proc. Natl. Acad. Sci. USA 108, 5348–5353.
Kaattari, S.L., Zhang, H.L., Khor, I.W., Kaattari, I.M., Shapiro, D.A., 2002. Affinity maturation in trout: clonal dominance of high affinity antibodies late in the immune response. Dev. Comp. Immunol. 26, 191–200.
Klemsz, M.J., McKercher, S.R., Celada, A., Van Beveren, C., Maki, R.A., 1990. The macrophage and B cell-specific transcription factor PU.1 is related to the ets oncogene. Cell 61, 113–124.
Lam, S.H., Chua, H.L., Gong, Z., Lam, T.J., Sin, Y.M., 2004. Development and maturation of the immune system in zebrafish, Danio rerio: a gene expression profiling, in situ hybridization and immunological study. Dev. Comp. Immunol. 28, 9–28.
Lamers, C.H.J., de Haas, M.J.H., van Muiswinkel, W.B., 1985. The reaction of the immune system of fish to vaccination: development of immunological memory in carp, Cyprinus carpio L., following direct immersion in Aeromonas hydrophila bacterin. J. Fish Dis. 8, 10.
Langenau, D.M., Feng, H., Berghmans, S., Kanki, J.P., Kutok, J.L., Look, A.T., 2005. Cre/lox-regulated transgenic zebrafish model with conditional myc-induced T cell acute lymphoblastic leukemia. Proc. Natl. Acad. Sci. USA 102, 6068–6073.
Langenau, D.M., Ferrando, A.A., Traver, D., Kutok, J.L., Hezel, J.P., Kanki, J.P., Zon, L.I., Look, A.T., Trede, N.S., 2004. In vivo tracking of T cell development, ablation, and engraftment in transgenic zebrafish. Proc. Natl. Acad. Sci. USA 101, 7369–7374.
Langenau, D.M., Keefe, M.D., Storer, N.Y., Guyon, J.R., Kutok, J.L., Le, X., Goessling, W., Neuberg, D.S., Kunkel, L.M., Zon, L.I., 2007. Effects of RAS on the genesis of embryonal rhabdomyosarcoma. Genes Dev. 21, 1382–1395.
Li, J., Barreda, D.R., Zhang, Y.A., Boshra, H., Gelman, A.E., Lapatra, S., Tort, L., Sunyer, J.O., 2006. B lymphocytes from early vertebrates have potent phagocytic and microbicidal abilities. Nat. Immunol. 7, 1116–1124.
Lieschke, G.J., Oates, A.C., Crowhurst, M.O., Ward, A.C., Layton, J.E., 2001. Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. Blood 98, 3087–3096.
Liu, K., Victora, G.D., Schwickert, T.A., Guermonprez, P., Meredith, M.M., Yao, K., Chu, F.F., Randolph, G.J., Rudensky, A.Y., Nussenzweig, M., 2009. In vivo analysis of dendritic cell development and homeostasis. Science 324, 392–397.
Lugo-Villarino, G., Balla, K.M., Stachura, D.L., Banuelos, K., Werneck, M.B., Traver, D., 2010. Identification of dendritic antigen-presenting cells in the zebrafish. Proc. Natl. Acad. Sci. USA 107, 15850–15855.
MacLennan, I.C., 1994. Germinal centers. Annu. Rev. Immunol. 12, 117–139.
Marianes, A.E., Zimmerman, A.M., 2011. Targets of somatic hypermutation within immunoglobulin light chain genes in zebrafish. Immunology 132, 240–255.
Marrack, P., Lo, D., Brinster, R., Palmiter, R., Burkly, L., Flavell, R.H., Kappler, J., 1988. The effect of thymus environment on T cell development and tolerance. Cell 53, 627–634.
Mathias, J.R., Dodd, M.E., Walters, K.B., Yoo, S.K., Ranheim, E.A., Huttenlocher, A., 2009. Characterization of zebrafish larval inflammatory macrophages. Dev. Comp. Immunol. 33, 1212–1217.
Mathias, J.R., Perrin, B.J., Liu, T.X., Kanki, J., Look, A.T., Huttenlocher, A., 2006. Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish. J. Leukoc. Biol. 80, 1281–1288.
Matsumoto, M., Lo, S.F., Carruthers, C.J., Min, J., Mariathasan, S., Huang, G., Plas, D.R., Martin, S.M., Geha, R.S., Nahm, M.H., Chaplin, D.D., 1996. Affinity maturation without germinal centres in lymphotoxin-alpha-deficient mice. Nature 382, 462–466.
Naik, S.H., Metcalf, D., van Nieuwenhuijze, A., Wicks, I., Wu, L., O'Keeffe, M., Shortman, K., 2006. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. Nat. Immunol. 7, 663–671.
Niess, J.H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B.A., Vyas, J.M., Boes, M., Ploegh, H.L., Fox, J.G., Littman, D.R., Reinecker, H.C., 2005. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science 307, 254–258.
Nussenzweig, M.C., Steinman, R.M., 1980. Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. J. Exp. Med. 151, 1196–1212.
Overland, H.S., Pettersen, E.F., Ronneseth, A., Wergeland, H.I., 2010. Phagocytosis by B-cells and neutrophils in Atlantic salmon (Salmo salar L.) and Atlantic cod (Gadus morhua L.). Fish Shellfish Immunol. 28, 193–204.
Page, D.M., Wittamer, V., Bertrand, J.Y., Lewis, K.L., Pratt, D.N., Delgado, N., Schale, S.E., McGue, C., Jacobsen, B.H., Doty, A., Pao, Y., Yang, H., Chi, N.C., Magor, B.G., Traver, D., 2013. An evolutionarily conserved program of B-cell development and activation in zebrafish. Blood 122, e1–e11.
Parichy, D.M., Ransom, D.G., Paw, B., Zon, L.I., Johnson, S.L., 2000. An orthologue of the kit-related gene fms is required for development of neural crest-derived xanthophores and a subpopulation of adult melanocytes in the zebrafish, Danio rerio. Development 127, 3031–3044.
Parra, D., Rieger, A.M., Li, J., Zhang, Y.A., Randall, L.M., Hunter, C.A., Barreda, D.R., Sunyer, J.O., 2012. Pivotal advance: peritoneal cavity B-1 B cells have phagocytic and microbicidal capacities and present phagocytosed antigen to CD4+ T cells. J. Leukoc. Biol. 91, 525–536.
Petrie-Hanson, L., Hohn, C., Hanson, L., 2009. Characterization of rag1 mutant zebrafish leukocytes. BMC Immunol. 10, 8.
Pozos, T.C., Ramakrishnan, L., 2004. New models for the study of Mycobacterium-host interactions. Curr. Opin. Immunol. 16, 499–505.
Ramiscal, R.R., Vinuesa, C.G., 2013. T-cell subsets in the germinal center. Immunol. Rev. 252, 146–155.
Reizis, B., Bunin, A., Ghosh, H.S., Lewis, K.L., Sisirak, V., 2011. Plasmacytoid dendritic cells: recent progress and open questions. Annu. Rev. Immunol. 29, 163–183.
Renshaw, S.A., Loynes, C.A., Trushell, D.M., Elworthy, S., Ingham, P.W., Whyte, M.K., 2006. A transgenic zebrafish model of neutrophilic inflammation. Blood 108, 3976–3978.
Rijkers, G.T., Van Oosterom, R., Van Muiswinkel, W.B., 1981. The immune system of cyprinid fish. Oxytetracycline and the regulation of humoral immunity in carp (Cyprinus carpio). Vet. Immunol. Immunopathol. 2, 281–290.
Roca, F.J., Ramakrishnan, L., 2013. TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. Cell 153, 521–534.
Sambrook, J.G., Figueroa, F., Beck, S., 2005. A genome-wide survey of major histocompatibility complex (MHC) genes and their paralogues in zebrafish. BMC Genomics 6, 152.
Sato, A., Figueroa, F., Murray, B.W., Malaga-Trillo, E., Zaleska-Rutczynska, Z., Sultmann, H., Toyosawa, S., Wedekind, C., Steck, N., Klein, J., 2000. Nonlinkage of major histocompatibility complex class I and class II loci in bony fishes. Immunogenetics 51, 108–116.
Saunders, H.L., Oko, A.L., Scott, A.N., Fan, C.W., Magor, B.G., 2010. The cellular context of AID expressing cells in fish lymphoid tissues. Dev. Comp. Immunol. 34, 669–676.
Schorpp, M., Bialecki, M., Diekhoff, D., Walderich, B., Odenthal, J., Maischein, H.M., Zapata, A.G., Boehm, T., 2006. Conserved functions of Ikaros in vertebrate lymphocyte development: genetic evidence for distinct larval and adult phases of T cell development and two lineages of B cells in zebrafish. J. Immunol. 177, 2463–2476.
Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., Prinz, M., Wu, B., Jacobsen, S.E., Pollard, J.W., Frampton, J., Liu, K.J., Geissmann, F., 2012. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336, 86–90.
Scott, E.W., Simon, M.C., Anastasi, J., Singh, H., 1994. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science 265, 1573–1577.

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010

Star, B., Nederbragt, A.J., Jentoft, S., Grimholt, U., Malmstrom, M., Gregers, T.F., Rounge, T.B., Paulsen, J., Solbakken, M.H., Sharma, A., Wetten, O.F., Lanzen, A., Winer, R., Knight, J., Vogel, J.H., Aken, B., Andersen, O., Lagesen, K., Tooming-Klunderud, A., Edvardsen, R.B., Tina, K.G., Espelund, M., Nepal, C., Previti, C., Karlsen, B.O., Moum, T., Skage, M., Berg, P.R., Gjoen, T., Kuhl, H., Thorsen, J., Malde, K., Reinhardt, R., Du, L., Johansen, S.D., Searle, S., Lien, S., Nilsen, F., Jonassen, I., Omholt, S.W., Stenseth, N.C., Jakobsen, K.S., 2011. The genome sequence of Atlantic cod reveals a unique immune system. Nature 477, 207–210.

Stavnezer, J., Amemiya, C.T., 2004. Evolution of isotype switching. Semin. Immunol. 16, 257–275.

Steinman, R.M., 2007. Dendritic cells: understanding immunogenicity. Eur. J. Immunol. 37 (Suppl. 1), S53–S60.

Steinman, R.M., Cohn, Z.A., 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J. Exp. Med. 137, 1142–1162.

Sultmann, H., Mayer, W.E., Figueroa, F., O'Huigin, C., Klein, J., 1994. Organization of Mhc class II B genes in the zebrafish (Brachydanio rerio). Genomics 23, 1–14.

Sunyer, J.O., 2013. Fishing for mammalian paradigms in the teleost immune system. Nat. Immunol. 14, 320–326.

Tamoutounour, S., Guilliams, M., Montanana Sanchis, F., Liu, H., Terhorst, D., Malosse, C., Pollet, E., Ardouin, L., Luche, H., Sanchez, C., Dalod, M., Malissen, B., Henri, S., 2013. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. Immunity 39, 925–938.

Trombetta, E.S., Mellman, I., 2005. Cell biology of antigen processing in vitro and in vivo. Annu. Rev. Immunol. 23, 975–1028.

Trump, G.N., Hildemann, W.H., 1970. Antibody responses of goldfish to bovine serum albumin. Primary and secondary responses. Immunology 19, 621–627.

van der Vaart, M., van Soest, J.J., Spaink, H.P., Meijer, A.H., 2013. Functional analysis of a zebrafish myd88 mutant identifies key transcriptional components of the innate immune system. Dis. Model Mech. 6, 841–854.

Van Voorhis, W.C., Valinsky, J., Hoffman, E., Luban, J., Hair, L.S., Steinman, R.M., 1983. Relative efficacy of human monocytes and dendritic cells as accessory cells for T cell replication. J. Exp. Med. 158, 174–191.

Viret, C., Janeway Jr., C.A., 1999. MHC and T cell development. Rev. Immunogenet. 1, 91–104.

von Bernuth, H., Picard, C., Jin, Z., Pankla, R., Xiao, H., Ku, C.L., Chrabieh, M., Mustapha, I.B., Ghandil, P., Camcioglu, Y., Vasconcelos, J., Sirvent, N., Guedes, M., Vitor, A.B., Herrero-Mata, M.J., Arostegui, J.I., Rodrigo, C., Alsina, L., Ruiz-Ortiz, E., Juan, M., Fortuny, C., Yague, J., Anton, J., Pascal, M., Chang, H.H., Janniere, L., Rose, Y., Garty, B.Z., Chapel, H., Issekutz, A., Marodi, L., Rodriguez-Gallego, C., Banchereau, J., Abel, L., Li, X., Chaussabel, D., Puel, A., Casanova, J.L., 2008. Pyogenic bacterial infections in humans with MyD88 deficiency. Science 321, 691–696.

Wang, J.H., Nichogiannopoulou, A., Wu, L., Sun, L., Sharpe, A.H., Bigby, M., Georgopoulos, K., 1996. Selective defects in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. Immunity 5, 537–549.

Ward, A.C., McPhee, D.O., Condron, M.M., Varma, S., Cody, S.H., Onnebo, S.M., Paw, B.H., Zon, L.I., Lieschke, G.J., 2003. The zebrafish spi1 promoter drives myeloid-specific expression in stable transgenic fish. Blood 102, 3238–3240.

Willett, C.E., Kawasaki, H., Amemiya, C.T., Lin, S., Steiner, L.A., 2001. Ikaros expression as a marker for lymphoid progenitors during zebrafish development. Dev. Dyn. 222, 694–698.

Wittamer, V., Bertrand, J.Y., Gutschow, P.W., Traver, D., 2011. Characterization of the mononuclear phagocyte system in zebrafish. Blood 117, 7126–7135.

Xu, Z., Parra, D., Gomez, D., Salinas, I., Zhang, Y.A., von Gersdorff Jorgensen, L., Heinecke, R.D., Buchmann, K., LaPatra, S., Sunyer, J.O., 2013. Teleost skin, an ancient mucosal surface that elicits gut-like immune responses. Proc. Natl. Acad. Sci. USA 110, 13097–13102.

Yang, D., Liu, Q., Ni, C., Li, S., Wu, H., Wang, Q., Xiao, J., Zhang, Y., 2013. Gene expression profiling in live attenuated Edwardsiella tarda vaccine immunized and challenged zebrafish: insights into the basic mechanisms of protection seen in immunized fish. Dev. Comp. Immunol. 40, 132–141.

Yang, F., Waldbieser, G.C., Lobb, C.J., 2006. The nucleotide targets of somatic mutation and the role of selection in immunoglobulin heavy chains of a teleost fish. J. Immunol. 176, 1655–1667.

Ye, J., Kaattari, I.M., Kaattari, S.L., 2011. The differential dynamics of antibody subpopulation expression during affinity maturation in a teleost. Fish Shellfish Immunol. 30, 372–377.

Yoder, J.A., Haire, R.N., Litman, G.W., 1999. Cloning of two zebrafish cDNAs that share domains with the MHC class II-associated invariant chain. Immunogenetics 50, 84–88.

Yona, S., Kim, K.W., Wolf, Y., Mildner, A., Varol, D., Breker, M., Strauss-Ayali, D., Viukov, S., Guilliams, M., Misharin, A., Hume, D.A., Perlman, H., Malissen, B., Zelzer, E., Jung, S., 2013. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity 38, 79–91.

Yoo, S.K., Deng, Q., Cavnar, P.J., Wu, Y.I., Hahn, K.M., Huttenlocher, A., 2010. Differential regulation of protrusion and polarity by PI3K during neutrophil motility in live zebrafish. Dev. Cell 18, 226–236.

Yoo, S.K., Huttenlocher, A., 2011. Spatiotemporal photolabeling of neutrophil trafficking during inflammation in live zebrafish. J. Leukoc. Biol. 89, 661–667.

Yuseff, M.I., Pierobon, P., Reversat, A., Lennon-Dumenil, A.M., 2013. How B cells capture, process and present antigens: a crucial role for cell polarity. Nat. Rev. Immunol. 13, 475–486.

Zakrzewska, A., Cui, C., Stockhammer, O.W., Benard, E.L., Spaink, H.P., Meijer, A.H., 2010. Macrophage-specific gene functions in Spi1-directed innate immunity. Blood 116, e1–e11.

Zhang, Y.A., Salinas, I., Li, J., Parra, D., Bjork, S., Xu, Z., LaPatra, S.E., Bartholomew, J., Sunyer, J.O., 2010. IgT, a primitive immunoglobulin class specialized in mucosal immunity. Nat. Immunol. 11, 827–835.

Zwollo, P., 2011. Dissecting teleost B cell differentiation using transcription factors. Dev. Comp. Immunol. 35, 898–905.

Please cite this article in press as: Lewis, K.L., et al. Perspectives on antigen presenting cells in zebrafish. Dev. Comp. Immunol. (2014), http://dx.doi.org/10.1016/j.dci.2014.03.010