

Specific function and modulation of teleost monocytes/macrophages: polarization and phagocytosis

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ABSTRACT

Macrophages exist in most tissues and play a variety of functions in vertebrates. Teleost fish species are found in most aquatic environments throughout the world and are quite diverse for a group of vertebrate animals. Due to whole genome duplication and environmental adaptation, teleost monocytes/macrophages possess a variety of different functions and modulations compared with those of mammals. A deeper understanding of teleost monocytes/macrophages in the immune system will not only help develop teleost-specific methods of disease prevention but will also help improve our understanding of the various immune mechanisms in mammals. In this review, we summarize the differences in polarization and phagocytosis of teleost and mammalian macrophages to improve our understanding of the various immune mechanisms in vertebrates.

Keywords: Teleost; Monocytes /Macrophages; Phagocytosis; Cytokine production; Comparative immunology

INTRODUCTION

Macrophages exist in most animal tissues, in which they play a variety of functions. They are known by different names in various groups, such as amebocytes, hemocytes, coelomocytes, granulocytes, monocytes, and macrophages, but have similar morphology and comparable functions (Bilej et al., 2000; Wiegertjes et al., 2016). Macrophages are best known for their role in immunity, as elucidated by Eli Metchnikoff in the late nineteenth century (Tauber, 2003). Several studies have highlighted the wide range of functions of macrophages in vertebrate biology, including systemic metabolism, cold adaptation, tissue homeostasis, and development (Okabe & Medzhitov, 2016; Wynn et al., 2013). The basic functions of macrophages in vertebrates are cytokine production and

phagocytosis (Hodgkinson et al., 2015). In both mammals and fish, monocytes give rise to macrophages during inflammatory conditions (Hodgkinson et al., 2015). Moreover, macrophage colony stimulating factor (CSF1) plays a crucial role in macrophage growth and differentiation in both mammals and fish (Hodgkinson et al., 2015). Teleosts are among the evolutionarily oldest vertebrates, possessing both innate and classical adaptive immune systems (Dickerson & Findly, 2017). Environmental factors have been shown to affect the immune system of teleosts (Makrinos & Bowden, 2016), which are widespread in most aquatic environments. Various immune genes in non-model teleosts have been identified, with transcriptome and genome development (Mackintosh & Ferrier, 2017; Qian et al., 2014; Shao et al., 2016), providing an opportunity to conduct studies on the adaptive evolution of the immune system. In this review, we focus on the differences in polarization and phagocytosis of teleost and mammalian macrophages, which should help in the development of a new perspective on macrophages and their role in adaptive evolution.

DEFINITION OF MONOCYTES/MACROPHAGES IN TELEOSTS

Monocytes/macrophages in mammals are an important component of the mononuclear phagocytic system, and play diverse roles during infection, inflammation, and tissue injury and repair (Okabe & Medzhitov, 2016). In mammals, protein markers are used to identify monocytes, macrophages, and dendritic cells by flow cytometry. Monocytes, which mainly

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exist in bone marrow, blood, and spleen, can further differentiate into inflammatory macrophages and dendritic cells during inflammation (Ray & Rai, 2017; Shi & Pamer, 2011). Their migration to tissues and differentiation into inflammatory macrophages and dendritic cells are determined by the inflammatory milieu and pattern recognition receptors on the cells (Murray, 2018). Macrophages reside in a variety of tissues, including lymphoid and non-lymphoid tissues. They are equipped with a broad range of pattern recognition receptors to facilitate cytokine production and phagocytosis during inflammatory responses (Franz & Kagan, 2017). Classical dendritic cells are specialized antigen processing and presenting cells that exhibit high phagocytic activity as immature cells and high cytokine-producing capacity as mature cells (Qian & Cao, 2018).

In teleosts, monocytes/macrophages have been identified in a variety of species, including rainbow trout (*Oncorhynchus mykiss*) (Leal et al., 2017), zebrafish (*Danio rerio*) (Yu et al., 2017), goldfish (*Carassius auratus* L.) (Hodgkinson et al., 2017), and ayu (*Plecoglossus altivelis*) (Lu et al., 2017). The human CD14 antigen is highly expressed in monocytes and to a lesser extent in macrophages (Shi & Pamer, 2011; Zhang et al., 2017). However, CD14 has not been identified in the teleost genome (Novoa et al., 2009). Hence, it is difficult to discriminate between teleost monocytes and macrophages based on protein markers using flow cytometry. Stafford et al. (2001) developed a method using cell-conditioned medium to obtain monocyte-like cells in rainbow trout. However, it is unclear whether monocytes can be identified from the culture of teleost monocytes/macrophages based on acute isolation using simple procedures, such as flow cytometry. Although dendritic-like cells have been identified in rainbow trout (Soletto et al., 2018), it is unclear whether such cells exist in other dominant teleost species. As monocytes and macrophages share similar characteristics, most teleost investigations have defined the adhered mononuclear cells of the kidney or head kidney as monocytes/macrophages or macrophages.

Phagocytosis is the defining characteristic of macrophages (large eaters), as classified by Metchnikoff (Tauber, 2003). However, most phagocytes among the peripheral blood mononuclear cells of teleosts are IgM⁺ B lymphocytes, not monocytes/macrophages (Li et al., 2006). Thus, morphological analysis, apart from phagocytosis index analysis, is necessary to identify the monocytes/macrophages in teleosts.

MACROPHAGE POLARIZATION AND CYTOKINE PRODUCTION

In vertebrates, the inflammatory response of macrophages plays an important role against pathogens (Geissmann et al., 2010; Ricci et al., 2018). Macrophage polarization against different inflammatory stimuli depends on environmental cues or pathophysiological conditions (Lawrence & Natoli, 2011). Classically activated macrophages (M1 type) are induced by lipopolysaccharides (LPSs), a major component of the outer membrane of gram-negative bacteria, and IFN- γ , and express

pro-inflammatory mediators. Conversely, alternatively activated macrophages (M2 type) are induced by IL-4 and IL-13, and express high levels of anti-inflammatory mediators (Shapouri-Moghaddam et al., 2018). Macrophage polarization is also regulated by soluble proteins, intracellular signals, and transcription factors. Galectin-dependent regulatory signaling stimulates M2-type macrophage polarization (Blidner et al., 2015). Toll-like receptor (TLR) signaling activates the signal transducer and activator of transcription (STAT) 1 protein to skew macrophage function towards the M1 type, whereas activation of STAT3 by IL-4 and IL-13 skews macrophage function towards the M2 type (Sica & Mantovani, 2012). Similarly, the ablation of protein kinase B α (PKB α /Akt1) or protein kinase B β (PKB β /Akt2) differentially affect macrophage polarization (Arranz et al., 2012). Tissue milieus with M1 type are biased towards cell-mediated cytotoxicity, whereas the term “M2 type” is used for a variety of conditions that inhibit M1 type (Yamaguchi et al., 2015). The immune milieus are skewed to M2 type in some tissues, like the gills in teleosts and uterus in pregnant mammals (Yamaguchi et al., 2015).

Monocyte/macrophage polarization has also been detected in teleosts (Wiegertjes et al., 2016). Cytokines participate in teleost monocyte/macrophage polarization, particularly IFN- γ and IL-4/IL-13 (Wiegertjes et al., 2016). Moreover, inducible nitric oxide synthase (iNOS) is a marker for M1 type and arginase 2 is a marker for M2 type in teleost monocytes/macrophages (Wiegertjes et al., 2016). In carp, the expression of pro-inflammatory cytokines IL-1 β , TNF- α , and CXCa, peak in peritoneal leukocytes 24 h after zymosan induction, whereas the expression of anti-inflammatory mediators IL-10 and arginase 2 peak 96 h and 168 h after zymosan induction, respectively (Chadzinska et al., 2008). This suggests that monocytes/macrophages display both classic- and alternative pathway-induced polarization upon immune stimulation *in vivo*. Monocyte/macrophage polarization in teleosts has also been investigated *in vitro*. LPSs from gram-negative bacteria are probably the best studied microbial stimuli for macrophage activation. However, the mammalian LPS receptor, TLR4, may not be functional in teleosts (Sepulcre et al., 2009). The presence of LPSs may be sensed by other mechanisms in teleosts, as LPSs are still thought to be important immune stimulators (Meng et al., 2012). Nitrite production in *in vitro* monocyte/macrophage culture is up-regulated after LPS stimulation in teleosts (Joerink et al., 2006). Other pro-inflammatory mediators, such as IL-1 β , IL-12, and iNOS are also up-regulated in LPS-stimulated teleost monocytes/macrophages (Joerink et al., 2006), suggesting that LPS induces an M1-type polarization in teleost monocytes/macrophages. In mammals, M2-type polarization is mainly induced by IL-4, IL-13, parasite infection, CSF1, TGF- β , and IL-10 (Sica & Mantovani, 2012). Apart from anti-inflammatory mediators, cAMP plays an important role in M2-type monocyte/macrophage polarization signaling (Bystrom et al., 2008). In teleosts, at least two IL-4/IL-13 genes exist (IL-4/13A and IL-4/13B), both with low homology to IL-4 and IL-13 (Ohtani et al., 2008). In goldfish, recombinant IL-4/13 has been found to induce arginase activity and down-regulate the nitric oxide

(NO) response in primary monocytes/macrophages (Hodgkinson et al., 2017), suggesting IL-4/13 functions to induce M2-type polarization in teleost monocytes / macrophages. In teleost carp (Joerink et al., 2006) and ayu (Chen et al., 2018), cAMP has also been employed to successfully induce M2-type monocyte/macrophage polarization.

Several new mechanisms for macrophage polarization have been identified recently. Mammals possess single IFN- γ molecules that are important in the activation of M1-type macrophages (Grayfer et al., 2018). Many teleosts have multiple IFN- γ molecules, some of which are elicitors of reactive oxygen species (ROS) but not NO, whereas others elicit NO but limited ROS (Grayfer et al., 2018). Although functional analogs of the mammalian M1/M2 macrophage subsets are present in teleosts, defining the regulatory mechanisms governing the polarization of these effector populations is a far more challenging goal (Grayfer et al., 2018). Many immune genes exist in two copies in the teleost genome due to genome duplication (Aghaallaei et al., 2010). These redundant genes may result in sub-functionalization, as in the case of European sea bass (*Dicentrarchus labrax*), in which different hepcidins exhibit different roles (Neves et al., 2015). We observed that ayu has two CXCR3 genes, which contribute to monocyte / macrophage polarization (Lu et al., 2017). In mammals, the chemokine receptor CXCR3 exists as a single gene, and is preferentially expressed on immune cells to aid in cell migration to the sites of inflammation (Bromley et al., 2008). Furthermore, CXCR3.1 (CXCR3b) and CXCR3.2 (CXCR3a) are found in zebrafish (*Danio rerio*), Japanese ricefish (*Oryzias latipes*), spotted green pufferfish (*Tetraodon nigroviridis*), ayu (*Plecoglossus altivelis*), and grass carp (*Ctenopharyngodon idella*) (Aghaallaei et al., 2010; Lu et al., 2017). However, more research is necessary to illustrate the teleost-specific mechanisms of monocyte/ macrophage polarization.

PHAGOCYTOSIS BY MONOCYTES/MACROPHAGES

Phagocytosis is an important cellular process for the induction of antimicrobial responses and regulation of adaptive immunity (Rieger et al., 2012). After phagocytosis, both teleost and mammalian macrophages show pro-inflammatory and homeostatic responses (Rieger et al., 2012). Mammalian neutrophils have the capacity to internalize apoptotic bodies, whereas teleost neutrophils do not possess the same activity (Rieger et al., 2012). Most studies have shown that in fish, monocytes, macrophages, and neutrophils are the main phagocytic cells, as found in mammals (Esteban et al., 2015). Furthermore, phagocytosis in teleosts has been observed in several other kinds of cells, including B-1 cells (Li et al., 2006), $\gamma\delta$ -T cells (Wan et al., 2016), and thrombocytes (Nagasawa et al., 2014). Although mammalian phagocytosis has also been observed in B-1 cells (Parra et al., 2012) and $\gamma\delta$ -T cells (Wu et al., 2009), its presence in thrombocytes seems unique to teleosts. It is well established that teleost

monocytes/macrophages generate reactive oxygen intermediates as part of antimicrobial defense, similar to that observed in mammalian macrophages (Hodgkinson et al., 2015). However, significant differences between goldfish and mice have been observed in response to pro-inflammatory and homeostatic internalization signals during phagocytosis of immune cells (Rieger et al., 2012). When European sea bass (*Dicentrarchus labrax*) monocytes/macrophages have been incubated with different pathogenic agents, different pathogens have been observed to have different effects on monocyte/ macrophage activity (Bennani et al., 1995). However, further investigation is necessary to illustrate the specific mechanisms of pathogen recognition and phagocytosis in teleosts.

Phagocytosis in mammals is triggered by pathogen recognition, which is a complex process involving a variety of pattern-recognition receptors. The main pattern-recognition receptors include lectin-like recognition molecules, C-type lectins, scavenger receptors, mannose receptors, CD14, and Toll-like receptors (Uribe-Querol & Rosales, 2017). In teleosts, the pathogen recognition mechanism is different from that of mammals. LPSs are sensed by a variety of pattern-recognition receptors (Ranf, 2016) present on the cell surface (Triantafilou & Triantafilou, 2002). Phagocytosis in macrophages is regulated by LPS recognition receptors, such as TLR4 (Lv et al., 2017) and CD14 (Lingnau et al., 2007). TLR4 was the first receptor identified to be involved in the recognition of LPSs. TLR4 must be associated with myeloid differentiation protein 2 (MD2) for interaction with LPSs, and activation of TLR4 is preceded by the transfer of LPSs to CD14 by an LPS-binding protein (Neyen & Lemaitre, 2016). However, CD14 and MD2 do not exist in teleost genomes (Iliev et al., 2005). Thus, it remains unclear how teleost monocytes/macrophages compensate for the CD14 function during phagocytosis of pathogens.

FUTURE DEVELOPMENTS

Teleost fish species are found throughout the world and are quite diverse for a group of vertebrate animals. Monocytes/ macrophages are the basic immune cells not only in mammals but also in teleosts. Monocytes/macrophages are easy to isolate, purify, and segregate, providing us with a useful tool for understanding the differences between the immune systems of mammals and teleosts. Although the basic function of monocytes/macrophages is similar in vertebrates, there are a variety of different points between mammals and teleosts (Figure 1). These differences have likely arisen from genetic factors, such as whole genome duplication, and environmental adaptations. Whole genome duplication in teleosts produces a variety of redundant genes, which may be sub-functional during adaptive evolution. Gene function in teleosts is modulated by environmental factors, such as salinity, hypoxic conditions, and temperature. Therefore, future investigations regarding the mechanisms of teleost monocyte/ macrophage polarization and phagocytosis under the influence of various environmental factors are necessary. A deeper understanding of the teleost immune system will not only help develop

teleost-specific methods of disease prevention but will also help improve our understanding of the various immune mechanisms within mammals.

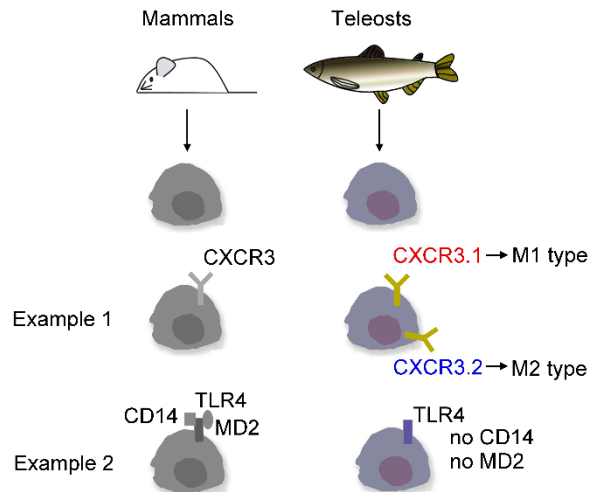


Figure 1 Differences in monocytes/macrophages between mammals and teleosts

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.J.L. and J.C. designed the study. J.C. and X.J.L. revised the manuscript. All authors read and approved the final version of the manuscript.

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