Teleostei Innate Immune System

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The innate immune system is the first line of defense in multicellular organisms. *Danio rerio* is widely considered a promising model for IIS-related research, with the most amount of scRNAseq data available among *Teleostei*.

Keywords: aquaculture ; drug development ; gene networks ; immunity

1. Introduction

The innate immune system (IIS) is a fundamental way of protecting multicellular organisms from a variety of pathogens and fighting against diseases [1]. This system originated approximately 700–800 million years ago and started with the first phagocytic cells of multicellular organisms [2]. Still, modern studies focus on using mice as a model for human disease, with a particular focus on the adaptive component of the immune system [3][4]. However, the impact of the IIS on certain diseases remains understudied. It is well known that the specific properties of the IIS could make a huge contribution to lifespan and resistance to diseases in mammals, foremost in the case of the naked mole-rat [5]. Spatial and single-cell transcriptomics (scRNAseq) datasets are especially valuable for IIS-related studies, because this system is highly decentralized. ScRNAseq technologies could be effectively used to uncover the properties of populations of teleost immune cells and their interactions during immune response [6].

The zebrafish, *Danio rerio*, is a comprehensively studied model object of fish genetics. The presence of an adaptive component of the immune system in adult zebrafish makes it a promising model for human diseases [7]. At the same time, the larval stages could be used as a model for the isolated IIS response [8]. However, *Danio rerio* remains underutilized in the context of IIS-related studies, e.g., host–microbe interactions [8] and human infections [9]. In recent years, we have seen a growing interest in the evolutionary and ecological aspects of the *Teleostei* immune system due to the growing need to control multiple diseases in aquaculture [10]. In this sense, teleost fish could become a hub taxon in studying the properties of IIS. Presently, more and more details are emerging about the evolution and organization of fish IIS. Obtaining new ScRNAseq data related to the IIS of fish could further extend the knowledge of the general aspects of the organization and functioning of innate immunity.

2. A Brief Overview of the *Teleostei* Innate Immune System

Danio rerio is the model organism of *Teleostei* used in many innate immunity studies [11]. The main regulatory factors of IIS [12] and immune cell isolation protocols [13] for zebrafish are well-known. There is a high similarity between teleosts and mammals' complementary systems [14], such as many common pattern recognition receptors [15] and downstream signaling components [16]. Also, homologues of mammalian NOD-like and Toll-like receptors are presented in the fish genome [17], as are many RIG-I-like receptors [16]. Petit et al. [18] identified several candidates for β-glucan receptors in the carp genome and emphasized the general similarity between mammals and fish in CLR-activating pathways. In addition, two homologues of Cyclic GMP-AMP synthase from the cGAS-STING DNA-sensing pathway were identified in zebrafish [19]. Moreover, the major histocompatibility complex (MHC) organization in fish resembles the mammalian one [20]. Murdoch and Rawls emphasized evolutionary conservatism in microbiota perception and response between fish and mammals, especially for microbiota-induced innate immune phenotypes [21]. Summarizing the facts above, there is strong evidence for significant homology in IIS organization between animals and fish.

Teleostei lack lymph nodes and bone marrow, but they have kidney marrow as a functional equivalent of human bone marrow [22]. Zebrafish have three main stages of hematopoiesis: **early-embryonic, embryonic, and adult** [23]. **Early-embryonic** hematopoiesis in zebrafish occurs in two waves: (1) the primitive wave 10–36 h post fertilization (hpf), where the posterior lateral-plate mesoderm (PLPM) forms primitive erythrocytes and neutrophils, and the anterior lateral-plate mesoderm (ALPM) gives rise to primitive macrophages and neutrophils; (2) the definitive wave (23–98 hpf), where PLPM cells pass through the transient stage (24–30) and form erythro-myeloid progenitors, and a subpopulation of cells from the ventral wall of the dorsal aorta (VDA) go through endothelial–hematopoietic transformation and form primitive multipotent

hematopoietic stem cells (HSCs) (26–54 hpf), part of which are released and form hematopoietic stem progenitor cells (HSPCs) [23]. **Embryonic** hematopoiesis occurs in caudal hematopoietic tissue (CHT) two days post fertilization (dpf), and through this period, HSCs and HSPCs are able to self-renew and differentiate. **Adult** hematopoiesis is occurring in the thymus at 3 dpf and in the pronephros at 4 dpf. The pronephros accommodates self-renewing and differentiating HSCs and HSPCs and plays the role of the main organ in adult hematopoiesis as mesonephros [23]. Therefore, there are differences in the origin and molecular properties of IIS cells between embryonal, larval, and adult stages of teleosts. Also, the activation of the adaptive component of the immune system of zebrafish occurs in a period 4–6 weeks after fertilization [24].

Teleosts produce all the main types of blood cells of IIS: **macrophages, granulocytes (neutrophils and eosinophils), dendritic cells, B lymphocytes, nonspecific cytotoxic cells, and mast cells** [25]. The key components of the IIS of fish are macrophages and neutrophils [26]. The general, up-to-date overview of the main components of the fish immune system is provided by Mokhtar and coauthors [27].

Macrophages are professional phagocytes that play an essential role in the regeneration processes of various tissues and organs (heart, fin, microglia, and others) [28]. Besides their roles in immune response, macrophages connect innate and adaptive components of the teleosts immune system, and their polarization into M1 or M2 types occurs under different stimuli [29]. There is emerging evidence that the metabolic reprogramming of macrophages in teleosts is similar to that in mammals: inflammatory macrophages (M1) are reprogrammed toward glycolysis, and anti-inflammatory macrophages (M2) are reprogrammed toward oxidative phosphorylation [30][31]. Fish macrophages in the liver play a crucial role in the immune response of this organ and could be easily visualized in real time using various fluorescent zebrafish lines, both in the adult and larval stages of development, for modeling various liver pathologies [32].

Neutrophils are important players in inflammatory processes against different pathogens in fish. The is clear similarity in the acute inflammatory responses of neutrophils between fish and mammals, but the huge reduction in neutrophil number in the circulating blood of fish compared with mammals was found by Havixbeck and Barreda [33]. Neutrophils are the main controllers of invasive infection and promoters of transformed cell proliferation [26].

Mast cells and **eosinophils** in fish are functionally similar to the mast cells of mammals, and an increase in the amount of these cells is usually detected in inflamed tissues [34]. Also, there is evidence of a difference between basophilic and eosinophilic components for various species of fish [34]. The importance of fish mast cells in immune responses and diseases was emphasized in the review by Sfacteria et al. [35]. Specialized defense dendritic cells are Langerhans cells; they recognize foreign antigens in skins and mucous membranes in various organisms, from fish to mammals [36]. These cells are likely to be able to activate T cells by expressing genes related to antigen presentation [37].

Teleost fish have four subpopulations of **B cells**. Three of them exclusively express surface immunoglobulins IgM, IgD, or IgT, and one subpopulation coexpresses surface IgM and IgD [38]. The fundamental mechanisms of immunoglobulin diversity in teleosts are similar to those in mammals [39]. It was found that mammalian B cells are stimulated in mucosa-associated lymphoid tissue (MALT), but in fish, MALT is subdivided into gut-associated, skin-associated, and gill-associated lymphoid tissue [38].

NK-like cells are able to kill altered virus-infected and foreign (allogeneic and xenogeneic) target cells [40]. A promising property of NK-like cells is their antitumor activity, which could be precisely studied at the single-cell level in real time [41]. **Nonspecific cytotoxic** (NCC) cells in fish possess killing activity against infected cells, tumors, and parasites [42]. Teng et al. [43] found significant proliferation of NCCs at the early stages of *N. seriolae* infection and CaNCCRP1 as a potential marker of nonspecific cytotoxic cells in fish.

There is limited information about the **eosinophils** and **basophils** of fish, since they are generally considered minor components of IIS. However, a recent study on the teleost fish *Takifugu rubripes* showed the existence of IgM on the surface of basophils. This fact is in favor of the activation of basophils in teleost in an Ab-dependent manner and means there is a possibility of interactions as antigen-presenters between basophils and T-cells [44].

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