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RESEARCH ARTICLE

Understanding fish B cell responses to combat infectious diseases

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Teleost fish possess all the necessary elements to mount an adaptive immune response, yet, the many physiological and structural differences between the mammalian and the teleost adaptive immune system, anticipate significant changes regarding how this response is coordinated and executed. As a result, the adaptive response in fish is often slower and weaker than that of mammals. B cells are key players in adaptive immune responses through the production of antibodies. Nonetheless, recent studies performed in mammals and other species including fish point to many additional functions of B cells within both the adaptive and the innate immune system, in many occasions taking part in the crosstalk between these two arms of the immune response. Furthermore, it should be taken into consideration that fish B cells share many functional and phenotypical features with mammalian innate B cell populations, also greatly conditioning their response to pathogens. Our knowledge regarding B cell function in fish has increased greatly in the past years, studies that have allowed us for example to identify different subsets of B cells, detect specific antibody-secreting cells or even establish the transcriptomic profile and the B cell receptor sequence of single cells in different stages of differentiation. In the current work, we will summarize what is currently known regarding fish B cells, knowledge that is essential for the future design of novel strategies to combat infectious diseases.

Introduction

The innate immune system englobes non-specific defense mechanisms not dependent upon previous pathogen encounter, which provide a first line of protection and constitute the basis of the immune response in invertebrates and lower vertebrates. In contrast, the adaptive immune system is stimulated by exposure to an antigen and increases in magnitude and defensive capacities with each successive exposure to this particular microorganism. Adaptive immunity involves either humoral or cellular responses. The humoral response is mediated by antibodies (immunglobulins, Igs) produced by B cells, which can recognize microbial antigens, neutralizing their infectivity and helping in their elimination. In contrast, cellular immunity is mediated by T cells that promote the destruction of pathogens that are localized inside cells such as viruses or intracellular bacteria, thus mediating the elimination of infected cells. Adaptive immunity constitutes the basis for vaccination, as what an effective vaccine aims for is to generate a memory response that will allow a rapid and strong reaction to a pathogen when encountered, by means of pre-exposing the host to an immunogenic non-harmful part of the

pathogen. Although effective vaccines, especially those against intracellular pathogens, should generate both memory B and T cells, in the current work, we will focus on what it is currently known regarding teleost B cells.

B cell responses in mammals

Antibodies, also termed immunoglobulins (Igs), are tetrameric molecules that include two identical heavy (H) chains and two identical light (L) chains. While IgH chains comprise one variable (V) domain and two to four constant (C) domains, IgL chains comprise one V domain and one C domain. V domains from paired IgH and IgL chains mediate antigen binding and account for the specificity of a given antibody. A recombined V_HDJ_H gene encodes each VH domain and results from the rearrangements of V, diversity (D) and joining (J) gene segments located in the IgH locus. These V(D)J recombination events sequentially involve random selection of individual gene segments, generation of double-strand breaks in each gene segment by rearrangement activation gene 1 (RAG1) and RAG2 endonucleases, deletion of the intervening DNA, and ligation of the remaining gene segments (Rodgers 2017). This complex process occurs in the bone marrow and generates antibody recognition diversity in an antigen-independent manner (Schroeder and Cavacini 2010).

In mammals, some B cell subsets such as B1 cells (mostly identified in mice) and marginal zone (MZ) B cells are capable of providing an early response to antigens in what has been commonly referred to as thymus independent (TI) responses. These TI responses do not require cooperation from T cells, but instead are co-activated by products secreted by cells of the innate immune system and by direct recognition of the pathogens at mucosal compartments (Cerutti, Puga, and Cols 2012). These cells, often considered components of the innate immune system, arise early during the ontogeny and play a key role in natural resistance, being essential for an early clearance of some types of pathogens. These innate B cells have a highly poly-specific (poorly mutated) B cell receptor (BCR) that can bind self-antigens or microbial products such as lipopolysaccharide (LPS), multivalent polysaccharides or large antigens with repetitive structures, being all of them TI antigens. Upon activation, these cells also differentiate to antibody secreting cells, although whether they reach a fully differentiated state (to plasma cells) or they remain as plasmablasts (that retain a proliferative capacity) is still under debate.

In contrast, conventional B2 cells (designated like this to contrast B1 cells) include MZ cells and a subset of B cells designated as follicular (FO) B cells that mediate the conventional mammalian humoral response. These FO cells are activated in sequential phases into what is commonly referred to as thymus-dependent (TD) responses:

- antigens (Ags) are presented to T helper (Th) cells by dendritic cells (DCs) in secondary immune organs (lymph nodes and spleen) and to B cells (naïve mature B cells co-expressing IgM and IgD on the cell surface) by subcapsular macrophages. These B cells become activated, loose surface IgD, migrate towards the T cell zone border and interact with Th cells.

- B cells then develop a secondary follicle and start a germinal center (GC) reaction. The GC reaction produces two main types of affinity matured B cells: (1) the memory precursors that can mediate the response to an Ag recall, and (2) post GC long-lived plasma cells that preferentially migrate and survive in the bone marrow. Within the GC, two different processes take place to increase the affinity of the produced antibodies, and adjust their effector functions to the situation that triggered the response. Thus, some activated cells begin to produce antibodies other than IgM in a process designated as class switch recombination (CSR). In CSR, the variable region of the antibody remains unchanged while the constant region of the heavy chain is replaced, going from IgM to either IgG (systemic responses), IgA (in mucosal infections) or IgE (in responses to allergies or parasitic infections). Furthermore, B cells also experience somatic hypermutation (SHM) randomly mutating the antigen-specific part of the Ig. Finally, those B cells with hypermutated Ig genes that produce antibodies binding antigens with higher affinity are preferentially expanded, leading to what is designated as affinity maturation.

Interestingly, an alternative non-GC clonal expansion of B2 cells may occur in the extrafollicular space of the secondary immune organ (extrafollicular responses). Extrafollicular B cell responses, as TI responses organized by innate B cell subsets, are all based on IgM production and provide the host with an initial protection until the GC reaction develops (Chappell et al. 2012).

Yet, mammalian FO B cell responses organized in GCs constitute the basis of adaptive immunity, being the optimal mechanism to select efficiently B cells expressing high affinity antibodies, and to control the development of memory cells. These B cell responses which may include B cell subsets expressing different Igs (IgM or switched Igs such as IgG, IgA or IgE) all originate from a few B cells that are subjected to clonal selection and expansion, CSR and somatic hypermutation, all being part of a single B cell lineage that experiences different differentiation steps.

Mucosal surfaces are in key in the immunological defense against pathogens since they constitute the barriers between the external media and the internal milieu. They comprise various lymphoid structures collectively referred to as mucosa-associated lymphoid tissue (MALT). The MALT includes the

gut-associated lymphoid tissue (GALT) that in mammals is organized in Peyer´s patches, mesenteric lymph nodes and isolated lymphoid follicles. Peyer´s patches are composed by aggregated lymphoid follicles containing B and T lymphocytes, as well as follicular DCs. Each follicle extends into a dome villus, that is covered by a follicle-associated epithelium (FAE) forming an interface between the GALT and the luminal microenvironment (Jung, Hugot, and Barreau 2010). Follicular TD-activation, clonal expansion and differentiation into T and B effector cells takes place in these inductive sites. Then, activated effector cells migrate to effector sites to carry out effector functions. Effector sites are present in all mucosal districts as a non-organized lymphoid tissue diffusely distributed throughout the lamina propria (LP). Here, cytotoxic T lymphocytes (CTLs) lyse infected cells and B cells differentiate into plasma cells that secrete large amounts of IgA, the predominant antibody isotype in intestinal secretions together with IgM, both of them transported across the epithelial cells by a polymeric Ig receptor (pIgR).

B cell responses in fish

Although teleost fish contain a fully functional adaptive immune system, the many structural differences when compared to its mammalian counterparts challenge well-established paradigms of systemic/central and mucosal immunity. In fish, the head kidney (HK) is the main hematopoietic organ in the absence of bone marrow, and has been considered thus far the main site for B cell development. Additionally, there are no lymph nodes, therefore the spleen constitutes the main secondary immune organ. Yet, the structure of the spleen seems to be disorganized when compared to mammals and no typical cognate GCs are ever formed. In mucosal surfaces, a lymphoid tissue can also be identified, but again it lacks the organized structure of mammalian MALTs and are mainly composed of B and T cells loosely scattered throughout the LP or as intraepithelial lymphocytes (IELs). For this reason, whether fish MALTs can be considered true MALTs were immune responses are induced is still a matter of debate (Salinas 2015).

Another main difference between the mammalian and the fish B cell system is that fish do not contain genes coding for the switched Igs (IgA, IgG or IgE) and rely exclusively on three Igs, IgM, IgD and IgT, a teleost specific Ig. Hence, no CSR has ever been reported in fish, given that IgT production is completely independent to that of IgM and IgD, as IgT generates diversity through different D and J segments. Consequently, two different B cell lineages are clearly identified in teleost fish, namely cells of the IgM/D lineage and IgT^+ B cells, being this a singular feature of the fish B cell system, completely different to the situation in mammals.

Thus, to date, B cells in teleost species have only been classified on the bases of Ig heavy chain (IgH) expression and whether different subsets of B cells according to functionality and location such as those described above

for mammals (B1, MZ, FO cells) exist, has never been established in fish. Nonetheless, recent studies from our group and others have identified that fish B cells in general share some attributes assigned in mammals to innate B cell populations such as B1 cells (Abós, Bird, et al. 2018; Scapigliati, Fausto, and Picchietti 2018). Thus, for example, fish B cells, similarly to mammalian B1 cells, have a strong phagocytic capacity (Li et al. 2006). Additionally, fish B cells are directly stimulated by pathogens, sensing them through innate receptors (Abós et al. 2013; Soleto et al. 2020). Similarly, they constitute one of the early responders to inflammation (Castro et al. 2017; Castro, Martínez-Alonso, et al. 2014). The fact that fish B cells retain these functions usually attributed to innate leukocyte populations, strongly suggests that fish B cells play an important role in the early stages of pathogen recognition and initiation of the immune response. It has to be taken into account that B cells are antigen-presenting cells, and that the strong phagocytic activity of fish B cells increases these capacities (Zhu et al. 2014).

B cell subsets in fish

Within what could be designated as the IgM/D lineage, like in mammals, IgM^+IgD^+ cells constitute the main B cell subset in systemic immune tissues (Simón et al. 2019). In these cells, IgM and IgD receptors are produced by alternative splicing of a long mRNA that includes the V_HDJ_H segment in addition to Cμ and Cδ and therefore express the same variable region (Geisberger, Lamers, and Achatz 2006). Upon activation by antigen, IgM^+IgD^+ B cells transcriptionally down-regulate surface IgD expression to become IgM^+IgD^- B cells, which have been shown in species such as rainbow trout to have increased IgM-secreting capacities and a transcriptional profile characteristic of plasmablasts/plasma cells (Morel et al. 2023). This transcriptional profile is quite similar to that of mammals and involves the up-regulation of transcription factors such as IRF4, the cytokine receptor BCMA (B cell maturation antigen) or Blimp1, for which 4 homologue genes have been identified in rainbow trout (Perdiguero et al. 2020). Yet whether these IgM-secreting plasmablasts constitute fully differentiated plasma cells is still not clear. Interestingly, given the lack of bone marrow in teleost fish, the HK has been identified as the main site for B cell maturation, since it contains B cells in different stages of maturation/differentiation including proliferating B cell precursors, plasmablasts and plasma cells (including long-lived plasma cells), from which the mature/naïve B cell migrate into other tissues via peripheral blood (Bromage et al. 2004).

Additionally, as it also occurs in mammals (Arpin et al. 1998; Koelsch et al. 2007; Shan et al. 2018), some IgM^+IgD^+ B cells lose surface IgM through a yet not well-defined non-canonical recombination event, generating IgM^-IgD^+ B cells. These cells that have the capacity to secrete IgD have been reported both in catfish blood (Edholm et al. 2010) and in some rainbow trout mucosal surfaces (Castro, Bromage, et al. 2014; Herranz-

Jusdado, Morel, Simón, et al. 2023; Perdiguero et al. 2019) such as gills, intestine and skin. Interestingly, this mucosal IgD, in contrast to splenic IgD, is clonally expanded and slightly mutated (Perdiguero et al. 2019). Furthermore, this secreted IgD was seen to establish a mutualistic relation with the intestinal microbiota (Perdiguero et al. 2019), suggesting an important role of IgD in mucosal homeostasis. Nonetheless, there are still many aspects of the role of these cells and that of secreted IgD in fish and mammals that need to be clarified.

Finally, most fish species (with a few exceptions), express IgT, a fish specific Ig (Hansen, Landis, and Phillips 2005). Given that IgT and IgM expression are mutually exclusive, IgT^+ B cells which do not co-express IgM or IgD constitute an independent cell lineage, producing antibodies with both a different IgH and also a different variable region. IgT^+ B cells, although found in most fish tissues, preferentially inhabit mucosal surfaces (Zhang et al. 2010). Interestingly, these cells represent the main responders to mucosal antigens such as those from commensal bacteria (Xu et al. 2013, 2016; Zhang et al. 2010). Additionally, IgT^+ B cells have been shown to preferentially respond to a diversity of pathogens and antigens in mucosal surfaces, whereas in these situations IgM responses seemed confined to systemic compartments (Xu et al. 2013, 2016; Zhang et al. 2010). Nonetheless, both IgM^+ and IgT^+ B cell responses have been observed in the spleen after systemic viral (Castro et al. 2013) or bacterial (Castro et al. 2019) infections and IgT responses were shown to be dominant in the kidney of fish infected with the parasite *Tetracapsuloides bryosalmonae* (Abós, Estensoro, et al. 2018). Similarly, both IgM^+ and IgT^+ B cells were shown to migrate to the heart in response to salmonid alphavirus infection (Bakke et al. 2020). Additionally, early IgM mucosal responses have also been described in fish (Herranz-Jusdado, Morel, Ordás, et al. 2023). Therefore, many aspects of how cells of the two lineages coordinate their responses to antigens both in systemic and mucosal compartments, are still unknown.

Conclusions

Although teleost fish are capable of mounting B cell responses to antigens, their response is quite different from that of conventional B2 cells in mammals, thereby strongly conditioning how they will react to vaccines. Although some lymphoid aggregates of B and T cells have been recently identified in fish that seem to be primitive structures in which these two populations cooperate and B cells are strongly activated (Shibasaki et al. 2023), these structures significantly differ from mammalian GCs. Additionally, given the lack of CSR in fish, and the poor affinity maturation rates usually reported (Ye, Kaattari, and Kaattari 2011), fish B cell responses seem to best resemble unswitched extrafollicular IgM responses. Nonetheless, these mechanisms are also capable of generating long-lived plasma cells exclusively based on IgM, implying that effective vaccination is fully

achievable in fish. However, it is quite essential that we fully understand how the adaptive immune system works in fish to be able to rationally design effective vaccines for each pathogen/ host.

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