Specific humoral immunity in Osteichthyes

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Abstract

The fish immune system is extremely complex and has considerable adaptive potential. In Osteichthyes, the system is formed by lymphopoietic organs which are important for the differentiation and maturation of the immune system cells. These organs include the anterior kidney (phronephros), the thymus, the spleen, the posterior kidney (mesonephros), and mucosa-associated lymphoid tissues (MALT). Apart from the lymphoctic organs and the MALT system, the immune system components include defensive cells and their products. Those identified in fish include, inter alia, monocytes/macrophages, melanomacrophages, neutrophilic granulocytes, thrombocytes, B cells, plasma cells, and T cells. The roles of the individual components of the organisation of the immune system, the organs, and lymphoid tissue as well as the constituents conditioning the innate and adaptive immunity mechanisms are considered equally important, especially in the context of functional interdependence. The progress in the exploration of the processes of specific humoral immunity in Osteichthyes and the possibilities of their practical application is increasingly promising in view of the expected need for protection of fish against diseases.

The paper discusses selected issues concerning recent knowledge about haematopoiesis of B cells, plasmablasts, plasma cells, and immunoglobulins (IgM, IgD, IgT/IgZ).

Key words: Osteichthyes, haematopoiesis, B lymphocytes, plasmablasts, plasmacytes, immunoglobulins.

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Introduction

The fish immune system is extremely complex and has considerable adaptive potential. In Osteichthyes, the system is formed by lymphopoietic organs which are important for the differentiation and maturation of the immune system cells. These organs include the anterior kidney (phronephros), the thymus, the spleen, the posterior kidney (mesonephros), and mucosa-associated lymphoid tissues (MALT) including gut-associated lymphoid tissue (GALT), skin-associated lymphoid tissue (SALT), gill-associated lymphoid tissue (GIALT), and nasopharynx-associated lymphoid tissue (NALT) [1]. MALT also includes lymphoid tissue clusters which have been identified in gills of salmon (Salmo salar) and described in recent years. Such clusters are referred to as ILT (interbranchial lymphoid tissue). As opposed to the other types of mucosa-associated lymphoid tissue that take the scattered form, ILT is organised tissue [2, 3]. Apart from the lymphoctic organs and the MALT system, the immune system components include defensive cells and their products. Those identified in fish include, inter alia, monocytes/macrophages, melanomacrophages, neutrophilic granulocytes, thrombocytes, B cells, plasma cells, and T cells [4-9].

The need to have necessary expertise on all the factors determining the development of the innate and adaptive immune response in Osteichthyes remains insatiable. The problem seems especially important, in particular from epidemiological and economic points of view, from the angle of the constantly high health risks to wild and farmed fish that are created by more or less severe environmental stressors as well as by genetic fingerprinting of pathogenic microorganisms that changes in fish habitats. Attempts to eliminate those risks and to counteract the often abrupt changes in the living conditions of fish in their natural and farmed environments generate wide-ranging and deep adaptation processes in these animals. The adaptation processes involve a number of physiological processes, including changes in the reactivity of the innate and adaptive immunity mechanisms [10, 11].

This paper presents selected aspects of specific humoral immunity in Osteichthyes, i.e. issues concerning haematopoiesis, B cells, plasmablasts, plasma cells, and immunoglobulins.

Haematopoiesis, B cells, plasmablasts, and plasma cells

In Osteichthyes, the role of a haematopoietic organ/tissue is played by the anterior kidney (phronephros, an
analogue of the K1 kidney segment in fish of the genus *Oncorhynchus*, where the kidney is divided into five segments, K1 to K5), deprived of nephrons and thus of the filtration and excretory functions that are fulfilled by the other kidney segments, including the posterior kidney *mesonephros*, an analogue of K5 [12, 13]. All haematopoietic differentiation pathways, i.e. erythropoiesis, myelopoiesis, and lymphopoiesis, have been identified in these fish [11, 12, 14]. The highest concentration of developmental forms of B cells conditioning specific humoral immunity is observed in the anterior kidney. In that kidney segment there are proliferating B cell precursors as well as plasmablasts and plasma cells that are classified as synthesising and antibody-secreting cells (ASC) [12, 15-17]. It is assumed [18] that the “haematopoietic” borderline between B cells and the cells of myeloid origin in fish is not very clear, and the cells of both origins demonstrate high functional flexibility. This is documented in lower vertebrates, including *Osteichthyes*, by the occurrence of a transitional stage in the evolution of B lymphocytes. In that stage, the cells that condition the innate/non-specific immunity mechanisms and are capable of phagocytosis evolve towards specialised forms, which are important for the development of specific immune response in higher vertebrates. This is expressed in, inter alia, the properties of IgM cells identified in fish, the properties of IgM cells present in the anterior kidney, and in peripheral blood (with a strongly marked ability to phagocytise) and of membrane (mIgM) and secretory (sIgM) cells (with a poorer ability to phagocytise). Kidneys in *Osteichthyes* are considered to be highly heterogeneous organs [18]. Apart from fulfilling the haematopoietic function, the anterior kidney also plays the role of an endocrine gland - the adrenal gland because it contains intrarenal adrenal cells, clusters of corticosteroid cells secreting the adrenocorticotropic hormone (ACTH) and cortisol as well as chromaffin cells secreting catecholamines [11, 19, 20]. In the posterior kidney, which, as opposed to the anterior kidney, fulfils filtration and excretory functions, there are in turn cells of the immune system, i.e. activated B cells, plasmablasts, and plasma cells. However, kidneys of *Osteichthyes* contain separate, location-dependent (K1-K5) phenotype patterns of B cells [12].

The common lymphocyte progenitor (CLP) is the earliest developmental stage of cells of lymphoid origin, including B cells. Their further development/differentiation stages include the pro-B stage and the pre-B stage (in sequence, large and small pre-B cells) as well as immature and mature B cells [12, 13]. Mature B cells migrate from the anterior kidney to the posterior kidney, as well as to the spleen and into the bloodstream. In the posterior kidneys and the spleens, the cells are subject to activation by, inter alia, melanomacrophages classified into antigen-presenting cells (APC) [21] and by T lymphocytes. This leads in the next differentiation stages to the formation of plasmablasts as well as of short-lived and long-lived plasma cells [12, 15]. As can be seen [12, 15, 22], short-lived plasma cells stay in the spleen, whereas long-lived cells migrate to the anterior kidney. This is the main area where mature plasma cells (MPC) with the Pax5$^+$ phenotype IgM$^+$ spread [23, 24]. Studies also show that both the spleen and the posterior kidney are peripheral/secondary lymphoid organs where antigen presentation takes place and where immune response develops, whereas the anterior kidney is a central/main lymphoid organ where B cells form and mature [12, 13, 16, 23]. Analysis of B cells by flow cytometry method confirms the fact that they are identifiable, in the particular developmental stages, based on a combined expression of specific transcription factors that play an important role in the development of B cells in vertebrates (inter alia: Ikaros, E2A, EBF1, Pax5, Blimp1, Xbp1) and class M, membrane and secretory immunoglobulins (IgM, mlgM, and sIgM, respectively) in different stages of immunity dependent on B cells [13, 16, 25-28]. There is evidence that B cells differ in the functions and demonstrate different properties, which are conditioned by, inter alia, the activity of transcription factors [23]. An early stage of the B cell development may be characterised by co-expression of the transcription factor EBF1 (early B-cell factor) and of the RAG1 gene (recombination activation gene) that is responsible for the activation of gene rearrangement for immunoglobulin receptor components, V-D-J, which code the B-cell receptor complex (BCR complex) recognising an antigen [13, 16]. MacMurray et al. [29] have reported of the factor EBF1 being expressed even at CLP and at a higher expression level, in large pre-B cells. In B cells in the subsequent developmental stages (small pre-B cells, immature, and mature B cells), the picture of combined expression of transcription factors manifests itself in a low level of expression of the factor EBF1 and in the absence of the factor RAG1 with simultaneous strong expression of the factor Pax5.PD (an isof orm of the Pax5 gene belonging to the Pax transcription factor family), which is the most important factor in the differentiation of B cells and the presence of mlgM [11, 29]. The transcription factor Pax5 and mlgM are also present in plasmablasts [24]. In contrast, the ultimately shaped plasma cells are characterised by the absence of the transformation factor Pax5 and of mlgM, with a simultaneous high level of IgM’s ability to secrete [23]. It is also useful to highlight the fact that the presence of Pax5.PD$^+$ B cells is very common and conservative in *Osteichthyes*. About 25% of the anterior kidney white blood cells (WBC) in different species of *Osteichthyes*, e.g. in rain brown trout (*Oncorhynchus mykiss*) [16], sockeye salmon (*Oncorhynchus nerka*) [30], or in common carp (*Cyprinus carpio*) [31] are Pax5.PD$^+$ cells. Furthermore, a small part of the B-cell population is distinguished by expression of IgT and/or IgZ (in terms of functions, IgT/Z correspond to IgA in mammals, also as a component of mucosal defence) or to IgD [11, 23, 32, 33]. According to Li et al. [18], the anterior kidney and the peripheral blood...
in rainbow trout (Oncorhynchus mykiss) contain IgM+ B cells with a phenotype profile defined by the expression of genes coding slgM as well as mlgM and mlgD. Those studies also show that the expression of mlgD genes was significantly higher in IgM+ cells demonstrating the ability to phagocytise. In that case, there were also IgM+ IgT+ cells that did not phagocytise. Their profile of expressed genes included mlgT, slgT, TCRα (T-cell receptor-α), TCRβ (T-cell receptor-β), CD8α (cluster of differentiation 8-α), and CSF-1R (colony stimulating factor 1 receptor), which probably, as suggested by Li et al. [18], make up a separate subgroup of lymphocytes, the properties of which correspond to those of IgM+ cells of lymphoid origin. There are equally large numbers of mature B Pax5.PD+, Xbp1+, and IgM cells in the spleen where the cells are activated by an antigen [12, 16, 24, 30]. After the antigen activation, there are also plasmablasts as well as short-lived and long-lived plasma cells as the ones that are concomitant with B Pax5.PD+ Xbp1+ cells and IgM-secreting cells [15, 22]. It is assumed that the activated B cells, Pax5+ IgM+ begin to synthesise and secrete small amounts of IgM, and this ability is enhanced in the subsequent differentiated forms of B cells, i.e. the proliferating IgM+ plasmablasts, and later in plasma cells, non-proliferating IgM+ Pax5+ [12, 15, 17, 21, 22]. Mature IgM+ B cells sensitive to LPS are also present in fish blood [13].

In Osteichthyes, just as in Chondrichthyes and mammals, the specific immunity mechanisms are marked by the presence of crucial molecules such as BCR (B-cell receptor), secretory immunoglobulins, TCR (T-cell receptor, CD4+ and CD8+), AID (activation-induced cytidine deaminase), cytokines, or major histocompatibility complex (MHC) class I and II antigens [1, 9, 34-37]. Immunoglobulins are the most important components of specific humoral immunity. To date, different heavy chain isotypes (IgH), including IgM, IgD/IgW, IgG, IgA, IgE, IgY, IgX, IgZ/IgT, IgF, and IgO have been identified in different vertebrate species [38]. For Osteichthyes, three isotypes, IgH, μ, δ, and τ/ζ, have been described. In this systematic group, these types form a basis for the division of immunoglobulins into three classes: IgM, IgD, and IgT/IgZ. The Hζ/ζ chain (Hζ-IgT) was first identified in rainbow trout, Oncorhynchus mykiss, whereas Hζ-IgZ was first identified in zebrafish, Danio rerio [17, 36, 39]. In Osteichthyes, these immunoglobulins are present in the plasma, skin, intestines, gills, and in the bile as well [40]. However, it must be taken into account that the presence of all the antibody classes named above has not been demonstrated in all Osteichthyes species [35]. The primary contact of Osteichthyes with an antigen generates in them, just as in species of higher systematic levels, synthesis and release of specific antibodies at a certain level, which is however lower than that in the case of secondary response developing as a result of new contact with an antigen. The secondary immune response evolves more quickly and more intensely, with a higher titre of specific antibodies [40].

**Immunoglobulins**

IgM is the first immunoglobulin identified in fish [41]. In B cells, it is in the form of a surface immunoglobulin (mlg), while in the serum and mucous membranes it is in the form of a secretory immunoglobulin (slg). slgM is a tetrameric molecule built of four monomeric units, each of which is built of two heavy chains (H) and two light chains (L). The H chain is composed of four domains, Cμ1-4, whereas mlgM has a monomeric form and is shorter than slg as a result of alternative splicing. It is characterised by the absence of the domain Cμ4 [17, 35, 42]. Even though serum IgM has been demonstrated to have a tetrameric form in most Osteichthyes, serum IgM has also been described to have a monomeric form. This form was found in Epinephelus itaita (family: Serranidae, genus: Epinephelus) and in Archosargus probatocephalus (family: Sparidae, genus: Archosargus) [42]. The quantity of IgM as natural antibodies in fish serum ranges between 800 and 9000 μg/ml. Their presence as specific antibodies developing in response to the presented antigen is observed even prior to immunisation [17, 35, 43-45]. These antibodies are also found in mucous membranes [1, 33, 45]. Just as in higher vertebrates, slgM in fish can be transported to the mucosal surface after they have formed a complex with the receptor for polymeric forms of immunoglobulins, plgR (polymeric Ig receptor). What needs to be highlighted is the fact that, as opposed to mammals in which a joining chain (J) is used for transporting IgM via plgR, the role of the J chain in fish is probably played (as no J-chain is present in the IgM structure) by an H chain that presumably has a binding module for plgR. Within the mucous membrane, IgM are also locally produced by plasma cells in response to an antigen [17]. Studies have shown [17] that just as in higher vertebrates, IgM fulfil essential effector defence functions in areas covered by both innate and adaptive immunity mechanisms. It has been demonstrated that IgM takes part in the killing of bacterial infectious agents, Aeromonas salmonicida, and the neutralisation of IHN viruses (infectious haematopoietic necrosis) and VHS viruses (viral haemorrhagic septicemia) as well as of parasites, Cryptobia salmositica [17]. The contribution of IgM to the activation of the complement, the opsonisation of bacterial antigens, inter alia Franciscella asiatica and Photobacterium damselae prior to the phagocytosis of these microorganisms by macrophages, have also been proven [35, 43, 46, 47].

IgD is identified in all vertebrates except for birds, and it is possible that IgD is equivalent to IgM in terms of phylogenesis [42, 48]. In Osteichthyes, IgD occurs in peripheral blood in the form of both mlg (in channel catfish – Ictalurus punctatus, B cells make up some 80%,...
whereas IgM+/IgD+ account for the remaining percentage) and sIg, in a concentration incomparably lower than IgM (in rainbow trout – Oncorhynchus mykiss – between 2 and 80 μg/ml) [32, 49]. There is evidence of sIgD in the anterior and posterior kidneys, the spleen, and the gills [35]. Also, Edholm et al. [49] claim, based on their research on channel catfish (Ictalurus punctatus), that IgD is in the character of an antigen-binding receptor both in the case of IgM/IgD+ cells with a high level of IgM and a low level of IgD in peripheral blood and in the case of IgM/IgD+ with a high level of IgD and the absence of IgM. It has been demonstrated that, just as in mammals, plasma cells of this immunoglobulin are present in gut-associated lymphoid tissue (GALT) and in the gills (GIALT). Additionally, there were very small numbers of plasma cells in peripheral blood (ca. 3%) [17, 32]. The significant role of this antibody isotype in the development of specific immune response in mucosa-associated lymphoid tissue is indicated by the presence of the V domain in the sIgD structure [32]. As studies on rainbow trout (Oncorhynchus mykiss) demonstrate, the sIgD level rises in GALT and GIALT in response to the immunisation with an attenuated strain of Flavobacterium psychrophilum [45]. The results of those studies confirm, as stated by Makesh et al. [45], the essential or simply specialised role of sIgD in mucosal immunity. The unique property of IgD is an H chain in the form of a hybrid created from the Cζ1 domain that is present in the IgD structure in all fish species, and from the Cδ domain that occurs in various numbers (from seven to as many as 16 Cδ domains) in different fish species [32, 35, 39, 50, 51]. In fish, an IgD H chain contains segments corresponding, one by one, to VDJ genes that codes the variable parts, in the fixed part, Cζ1 linked to a variable number of the Cδ domains, depending on fish species and the transmembrane part. IgD molecules in fish lack a hinge region [42].

IgT/IgZ is the Ig class that has been most recently found in such species of Ostechthybes as zebrafish (Danio rerio) [50, 52], rainbow trout (Oncorhynchus mykiss) [53, 54], Atlantic salmon (Salmo salar) [55], three-spined stickleback (Gasterosteus aculeatus) [56], and common carp (Cyprinus carpio) [57], in which the genome order, structure, and biochemical properties of these proteins have been identified. Research on rainbow trout (Oncorhynchus mykiss) has shown that IgT take part in the development of an immune reaction in response to bacterial and viral infections, and to DNA inoculations. These immunoglobulins also play an important role in the protection of the gills (GIALT), the skin (SALT), and of the gut mucosa (GALT) [45, 54, 58, 59]. The structure of the domains Cζ1/ζ4 varies in different fish species. In rainbow trout and zebrafish, IgT/IgZ have four domains, Cζ1-4 and Cζ41-4, respectively, whereas in tiger puffer (Takifugu rubripes; synonym: Fugu rubripes) they only have two domains, Cζ1ζ4 which correspond to Cζ1ζ1 and Cζ1ζ4, respectively, in zebrafish. In three-spined stickleback, they have three ζ domains and no Cζ2 domain among those identified in rainbow trout. Whereas in common carp, besides a subclass of IgZ1, there is a subclass of IgZ2 and chimeric IgM-IgT where the H chain is built of two domains, i.e. Cμ1 and Cζ4 (similar to Cμζ4 in rainbow trout) [17, 35, 57, 60]. Moreover, three subclasses of IgT, namely IgT1(τ1), IgT2 (τ2), and IgT3 (τ3) [54], have been identified in rainbow trout serum based on an analysis of CH domains using the LC-MS/MS technique (liquid chromatography-tandem mass spectrometry). The picture reflecting the immune reactivity related to IgT/Z is not always homogenous and thus not unambiguous. Studies carried out on rainbow trout during a parasitic invasion in its guts have demonstrated, for example, an increase in the number of IgT+ B cells with an unchanged number of IgM+ B cells and a comitant similar picture of the changes in the Ig levels in the gut mucosa and an increase in the quantity of IgT with no changes in the quantity of IgM. Furthermore, the IgT concentration in the gut mucosa 7.1 μg/ml was twice as high as the level of these Ig in serum [59]. IgT B cells in rainbow trout account for between 16 and 28% of the total number of these cells identified in the peripheral blood, the spleen, the anterior kidney, and in the body cavity, whereby the presence of IgM+ B cells absolutely dominates. The percentage shares of both the cell types in gut-associated lymphoid tissue (GALT) are completely different, shaped with values 54.3% IgT+ and 46.7% IgM+, respectively. IgT/Z is considered the most important among Ig within mucosa-associated lymphoid tissue [17, 54, 59]. Studies show that IgT in rainbow trout serum has a monomeric form (IgM has a polymeric form), whereas in the gut mucus, it is in a polymeric (tetrameric) form, just as IgM, and the transport of IgT from the serum to the gut mucosa can take place with the participation of the receptor for polymeric forms of immunoglobulins (pIgR) [59]. Similar results have been obtained by the team of Xu et al. [58] for rainbow trout affected by an invasion of freshwater ich (Ichthyophthirius multifilis). These fish were studied for, inter alia, the presence of IgT+ B cells and of polymeric IgT in skin-associated lymphoid tissue (SALT).

Conclusions

The exploration of the issues addressed in this paper should be judged as very dynamic and advanced in terms of methods and techniques. The progress in the exploration of the specific humoral immunity mechanisms in Ostechthybes and the possibilities of their practical use is increasingly promising in view of the expected need for protection of fish against diseases. The observed picture of specific humoral immunity in Ostechthybes is very complex and seems to be a combination of immune elements with high adaptive potential and an ability to protect the body of these animals.
The authors declare no conflict of interest.

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