B cells and their role in the teleost gut

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ABSTRACT

Mucosal surfaces are the main route of entry for pathogens in all living organisms. In the case of teleost fish, mucosal surfaces cover the vast majority of the animal. As these surfaces are in constant contact with the environment, fish are perpetually exposed to a vast number of pathogens. Despite the potential prevalence and variety of pathogens, mucosal surfaces are primarily populated by commensal non-pathogenic bacteria. Indeed, a fine balance between these two populations of microorganisms is crucial for animal survival. This equilibrium, controlled by the mucosal immune system, maintains homeostasis at mucosal tissues. Teleost fish possess a diffuse mucosa-associated immune system in the intestine, with B cells being one of the main responders. Immunoglobulins produced by these lymphocytes are a critical line of defense against pathogens and also prevent the entrance of commensal bacteria into the epithelium. In this review we will summarize recent literature regarding the role of B-lymphocytes and immunoglobulins in gut immunity in teleost fish, with specific focus on immunoglobulin isotypes and the microorganisms, pathogenic and non-pathogenic that interact with the immune system.

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1. Introduction

It is generally accepted that host mucosal immunity is characterized by tolerance rather than responsiveness (Arrieta and Finlay, 2012; Salinas and Parra, 2015). To maintain the integrity of the body, the host has developed a number of chemical and physical barriers armed with immune potential, which is hypothesized to have been driven by microbial colonization as a contributing factor in the evolution of the adaptive immune system (Lee and Mazmanian, 2010; McFall-Ngai, 2007). Aquatic environments provide an ideal setting for the growth of a variety of microorganisms that are in continuous contact with the mucosal surfaces of the fish body (Gómez et al., 2013). Thus, the intestine is combining two confounding functions, facilitating the absorption of nutrients, while resisting and inhibiting the breach of potential pathogens through its epithelium (Sommer and Bäckhed, 2013). In addition, due to the large microbial community residing in the gut, homeostasis maintenance is critical for the correct immune function of this mucosal tissue. During the last decade, we have witnessed a change in the paradigm, understanding that under normal conditions, the vast majority of microorganisms which are in contact with mucosal surfaces do not pose a threat, but positively contribute to host physiology (Arrieta and Finlay, 2012; Sommer and Bäckhed, 2013).

Together with cartilaginous fish, teleost fish are the earliest living organisms with an adaptive immune system based on B and T cell receptors. Thus, fish not only have most of the elements of the innate immune system of higher vertebrates, but also possess thymus, spleen, major histocompatibility complex class I and II, and T and B cells among the most important components of the adaptive immune system (Sunyer, 2013). Until recently, IgM had been considered the only immunoglobulin class responding to pathogenic challenge in systemic and mucosal compartments of teleost fish (Sunyer, 2013). However, the discovery of a novel immunoglobulin class (IgZ/IgT) in zebrafish (Danilova et al., 2005) and rainbow trout (Hansen et al., 2005) together with the finding that it played a specialized role in gut mucosal immunity (Zhang et al., 2010), triggered a renaissance in the study of teleost mucosal immunology. Later studies demonstrated that IgT plays also a key role in teleost skin and gill mucosal immune responses (Xu et al., 2016; Z. Xu et al., 2013). Such studies revealed that many fundamental mechanisms protecting mucosal surfaces evolved very

similarly in both mammals and fish (Sunyer, 2013).

In this review, we aim to explore further the current findings on the role of B lymphocytes in the intestine of teleost fish. First, we will review the anatomy of the intestinal immune system. Later we focus on the available information regarding gut B cells and immunoglobulins of teleost fish. Thereafter, we concentrate our attention on gut mucosal B cells responses to pathogens, followed by responses to probiotics and the microbiota. We later explore the possible parallels between fish and mammalian gut immune responses, and we identify areas in which studies in teleosts may shed light into unresolved paradigms of mammalian gut mucosal immunity. Together, the reviewed and future studies on fish intestinal immunity will provide crucial information for the formulation of fish vaccines and will also aid in the identification of fundamental mechanisms and principles underlying the protection of mucosal surfaces that are common to all jawed vertebrates.

2. The anatomy of the intestinal immune system

2.1. The anatomy of the mucosa-associated lymphoid tissue (MALT)

In order to keep homeostasis throughout evolution, the mucosal barriers have developed an immune system armed with both cellular and humoral defenses, protecting the organisms from the continuous bombardment of microbes and antigens. These mucosa-associated lymphoid tissues (MALT) harbor immune cells and produce humoral mediators, such as cytokines or immunoglobulins, among many others (Rombout et al., 2011, 2014; Salinas et al., 2011). In teleost, four different MALTs have been thus far described depending on their localization: SALT (skin-associated lymphoid tissue), GIALT (gill-associated lymphoid tissue), NALT (nasopharynx-associated lymphoid tissue) and GALT (gut-associated lymphoid tissue) (Gómez et al., 2013; Salinas and Parra, 2015; Salinas et al., 2011; Tacchi et al., 2014).

In higher vertebrates, the mucosal compartments can be divided into inductive and effector sites. The first are involved in the sampling of the antigen and stimulation of cognate naive T and B lymphocytes, while in the latter, the effector cells perform their action (i.e., antibody production) (Brandtzaeg et al., 2008). The inductive sites comprise the so-called organized MALT, such as the Peyer's patches (**PP**), mesenteric lymph nodes (MLN) and the isolated lymphoid follicles, in addition to the mucosa-draining lymph nodes. All these structures resemble classical lymph nodes, with T-cell zones intervening between the B-cell follicles, providing the anatomical, physiological and immunological basis for the maturation of antibody responses. On the other hand, the effector sites consist of distinct compartments including the lamina propria (LP) or the intraepithelial lymphocytes (IEL). Since the lymphocytes in LP and IEL are not organized, the tissue that comprises them is commonly termed as diffuse MALT. It is worth pointing out that while considered an effector site, the LP is also vital for the expansion of B cells and their differentiation into plasma cells (Brandtzaeg et al., 2008; Garside et al., 2004; Mason et al., 2008; Rombout et al., 2011; Salinas, 2015).

In teleost, organized lymphoid structures such as PP or MLN are missing, hence, the diffuse MALT may combine both inductive and effector functions, although that remains to be demonstrated (Table 1). Interestingly, the teleost interbranchial lymphoid tissue (ILT) was initially proposed as a possible exception that could represent an organized structure of teleost MALT (Aas et al., 2014; Gómez et al., 2013; Koppang et al., 2010; Salinas et al., 2011), however, this notion was reexamined and recently the ILT has been classified as a diffuse lymphoid tissue (Dalum et al., 2015). Importantly, it has been established that PP are not absolutely necessary for IgA production in mice (Lorenz and Newberry, 2004; Tsuji et al., 2008) which occurs also extrafollicularly. From that perspective, activation of B cells and mucosal immunoglobulin production in teleosts occurs extrafollicularly in the MALTs, as teleost fish develop pathogen-specific immunoglobulin responses at their mucosal surfaces despite the lack of organized lymphoid structures (Parra et al., 2015; Rombout et al., 2014; Salinas and Parra, 2015; Xu et al., 2016; Z. Xu et al., 2013).

2.2. Gut-associated lymphoid tissue (GALT)

Teleosts encompass more than 30,000 species, in comparison to approximately 5700 species of mammals and 10,000 species of birds. Naturally, there is a wide range of ecologies and diets within the group that is reflected in the organization of the gastrointestinal system. For example, the stomach develops in 85% of bony fish species, while the rest, including cyprinids, never develop a stomach and lack a region with low pH and predigestion (Balon, 1975). Additionally, depending on the fish species, important differences exist in the length of the intestine and the presence and number of pyloric caeca as well as intestinal loops and valves (Evans et al., 2014). As in mammals, immunological differences can be found along the different segments of the teleost gastrointestinal tract. In nearly all investigated species, the intestine can be subdivided in three segments based on the microscopical anatomy of their mucosa: i) the first part of the intestine (foregut, anterior gut), where enterocytes are considered absorptive cells; ii) the second segment (posterior gut), with enterocytes characterized by large supra-nuclear vacuoles, irregular microvilli zone and high pinocytotic activity and iii) the hindgut, where the enterocytes were proposed to play mainly osmoregulatory function (Rombout et al., 2011). A number of observations have been made with respect to the uptake and processing of antigens along these segments, indicating a principal role of the second segment in transport of antigens to the local and systemic lymphoid tissues (Rombout and van den Berg, 1985; Rombout et al., 1989, 2011). However, despite these findings, the detailed knowledge about the distribution of various lymphocyte subsets and secretory immunoglobulins in different proportions of the gut is scarce (Salinas and Parra, 2015; Salinas, 2015; Salinas et al., 2011). Regardless of these differences, the structure of GALT can be very similar among various fish species, although important anatomical differences exist in the gut among carnivorous, omnivorous and herbivorous fish which may be reflected also in differences in their GALTs (German and Horn, 2006).

As previously mentioned for MALT, the level of organization in GALT is less complex than in mammals, and lymphoid cells are more diffusely distributed as fish lack organized lymphoid structures. Similar to higher vertebrates, leukocytes can be found in two extrafollicular compartments: the LP, which harbors a variety of immune cells, including macrophages, granulocytes, lymphocytes and plasma cells; and the intraepithelial compartment that contains mostly T cells and very few B lymphocytes (Rombout et al., 2011; Salinas and Parra, 2015; Salinas et al., 2011). Despite being separated only by a thin basement membrane, the LP and the epithelium form very distinct immunological compartments (Mowat and Agace, 2014).

The uptake of antigen in the teleost intestine has been a subject of controversy. It has been proposed that enterocytes can uptake certain types of antigen, such as ferritin (Rombout and van den Berg, 1985). On the other hand, it has been suggested the presence of M cell-like cells, displaying morphological similarities with mammalian M cell (Fuglem et al., 2010). These cells, located in the posterior gut of salmonids, were proposed to represent evolutionary predecessor of mammalian M cells and were shown to take up bovine serum albumin (BSA) (Fuglem et al., 2010). Moreover, as demonstrated in cyprinids and salmonids, large intraepithelial