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Adaptive immune responses at mucosal surfaces of teleost fish

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ABSTRACT

This review describes the extant knowledge on the teleostean mucosal adaptive immune mechanisms, which is relevant for the development of oral or mucosal vaccines. In the last decade, a number of studies have shed light on the presence of new key components of mucosal immunity: a distinct immunoglobulin class (IgT or IgZ) and the polymeric Ig receptor (pIgR). In addition, intestinal T cells and their putative functions, antigen uptake mechanisms at mucosal surfaces and new mucosal vaccination strategies have been reported. New information on pIgR of Atlantic cod and common carp and comparison of natural and specific cell-mediated cytotoxicity in the gut of common carp and European seabass, is also included in this review. Based on the known facts about intestinal immunology and mucosal vaccination, suggestions are made for the advancement of fish vaccines.

1. Introduction

Aquaculture is a fast-growing food producing sector, and health management of the cultured species is critical for the sustainable growth of the industry. In this context, mucosal health of fish should be given prime importance as mucosal surfaces like the skin, the gills, the gut and the urogenital system constitute the first line of defence. The importance of mucosal barriers in aquatic animals is far more than those of their terrestrial counterparts as the aquatic species are continuously interacting with the microbiota in their environment. Over the last decades, efforts have been made to gain a better understanding of mucosal immune system, which in turn helps to develop vaccination strategies aimed at maximizing mucosal and consequently organismal health.

Vaccination is the most-appropriate method for the control of disease-causing pathogens from the economic, environmental and ethical point of view. At present, fish are commonly vaccinated by injection or immersion methods. Injection route is in general very effective, but it is labour-intensive and only practiced for high-value species like Atlantic salmon, *Salmo salar*. All life stages are prone to diseases, especially the early phases during which disease-related mortality frequently occurs. In farms, the young animals are subjected to immersion vaccination since it is not feasible to inject them individually. Novel vaccination methods that are cost-effective, simple, effortless, and less stressful to animals of all stages including young fish should be developed for aquaculture. The ideal technique that fulfils these criteria is oral vaccination (via feed), although this delivery route is not commonly used by the industry [1–4]. Modern tools such as nano-technology, which can be used to manipulate vaccines' size, cell-targeting and amount, may be adopted in aquaculture too [5].

More knowledge on both the antigen delivery and the mucosal immune defence systems, in particular on the mucosal adaptive immune responses in fish, should be generated. Peyer's patches, antigen transporting M cells, IgA- and the IgM-joining J chain – all the essential components of the mammalian mucosal immune system – are not yet reported in teleost fish [2]. The first inferences on local and/or mucosal responses of a variety of fish species were based on the detection of specific antibodies in mucosal secretions after intestinal [6–11] or immersion [12–15] immunisations. Nevertheless, upon systemic immunisation these specific mucosal antibodies were not or hardly detected. This differential generation of specific antibodies and the new information on specific antibody-producing cells at mucosal sites after intestinal [3,11] or immersion [14,15] vaccination inspired many scientists to study mucosal structures in different teleosts. The present review focuses on the mucosal adaptive immune system in fish. In fact, it is rather surprising that after the first publication on successful oral vaccination of rainbow trout, *Oncorhynchus mykiss* in 1942 [16] not much information on mucosal immunology in fish has been

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gathered compared to the knowledge on the mammalian mucosal immune system. For instance, concrete evidence on the existence of a common mucosal immune system and a separate mucosal immunoglobulin class or isotype has not yet been reported.

This review gives an insight into antigen uptake at the mucosal surfaces and subsequent local responses, the transport of immunoglobulins to mucosal surfaces by the polymeric Ig Receptor (pIgR) and its role in immune defence. Further, the possible functions of the abundant number of intraepithelial lymphocytes (mainly T cells) in the mucosal epithelia and the induction of oral tolerance in fish are also described. In addition, the significance of mucosal vaccination is summarized.

2. Mucosal *vs* systemic antigen responses

The most commonly used fish vaccination methods are injection [intraperitoneal (ip) or intramuscular (im)] and immersion (bath or spray). Besides these methods, antigens could be delivered via feeds – oral vaccines. The ip or im injections can be considered as systemic vaccinations since they produce only internal immune responses that are easily detectable in blood. In mammals, ip injection has also been claimed as a suitable priming route prior to oral vaccination [17]. In fish, ip injection can induce a certain degree of mucosal responses [18]. Immersion vaccination of fish, on the other hand, leads to uptake by the skin, the gills and the gut (after drinking) [19], subsequently inducing local responses. It has been reported that a hyperosmotic stressor, applied ahead of the immersion vaccination, brings about better uptake and higher responses, mostly at the mucosal surfaces [13]. Nevertheless, it is necessary to discover appropriate adjuvants that can reduce the amount of antigens required for mucosal vaccination. In fact, although many mucosal adjuvants for fish have been patented (see http://www.patentfish.com/as-mucosal-adjuvants), not many are being used for practical purposes.

In mammals, exposure of mucosal surfaces to antigens results in the secretion of antigen-specific IgA at these locations. Mammals have a common mucosal immune system, in which stimulation of one epithelium can also give rise to specific IgA or IgM responses in other mucosal organs, aided by the so-called systemic and mucosal homing receptors on immune competent cells [20,21]. It is not yet clear if fish possesses a common mucosal system or not. Till now specific homing of mucosal leucocytes has not been clearly detected [2,3], although suggestions on a homing model have been made by Fillatreau et al. [22]. However, evidences indicate induction of specific antibodies in the skin mucus, but not in the serum, following oral vaccination [7,8]. Orally administered antigens are taken up and transported via the end gut (the so-called 2nd segment), and if an adequate amount of antigen reaches this segment, local as well as systemic antibody responses are induced in fish [8]. On the other hand, when antigens are delivered anally they reach the 2nd segment immediately, and, therefore, even a small amount of antigen is sufficient to evoke systemic responses and memory formation [8,9]. Mucosal vaccines can be effective immune stimulators only if the antigens can reach the correct inductive sites and do not induce oral tolerance as suggested by Kim and Jang [23]. In addition, the efficacy of these vaccines in fish needs to be confirmed through pathogen challenge studies.

3. Mucosal antibodies

The spatial and quantitative differences in generation of specific antibodies in fish strongly suggest that differences exist between mucosal- and systemic-derived antibodies. Such differences were first reported in 1981 by Lobb and Clem [24], based on the presence of secretory component bound to dimeric Ig molecules in the skin mucus of sheepshead, *Archosargus probatocephalus*. A decade later, differential binding of monoclonal antibodies (mAb) to mucosal- and serum-derived IgM (mainly tetramers and dimers) was described in common carp, *Cyprinus carpio* [25]. The mAb (WCIM) derived from the skin mucus IgM recognized IgM heavy (H) chain of the skin mucus of common carp, but not that of the serum; strong and specific immunohistochemical reactions were also observed at mucosal Ig-localised sites such as the bile capillaries, ducts and the skin epithelium [25]. On the contrary, another mAb (WCI12), which is derived from serum IgM and that recognizes both H chains could be used for the detection of mucosal responses after intestinal and immersion immunisation, although it had a lower affinity for mucus IgM.

A new type of immunoglobulin H chain class has been reported in fish. In zebrafish, *Danio rerio* [26], common carp [27], mandarin fish, *Siniperca chuatsi* [28] and grass carp, *Ctenopharyngodon idella* [29] it is called IgZ, but in rainbow trout [30], Atlantic salmon [31] fugu, *Takifugu rubripes* [32], three spined stickleback, *Gasterosteus aculeatus* [33] and two Perciform species [cf [34]] it is termed IgT. The IgT in rainbow trout was suggested to have a role in mucosal immunity [34,35]. Among the two IgZ isotypes in carp, IgZ2 has a preference for mucosal tissues, while IgZ1 is associated with systemic organs [36]. IgZ2 appears to be a chimeric form having both μ1 and ζ4 domains, and trout IgT lacks this μ1 domain [22].

In addition to IgM and IgT/Z, IgD has also been described in a variety of teleosts [37–43]. Although it is known that IgD can be secreted [43], its involvement in mucosal responses has not been clarified. Histochemical observations on the digestive tract of rainbow trout [44] have revealed the preference of IgM+ cells in the lamina propria and IgT+ cells in the epithelium. These data indicate that the intraepithelial lymphocytes (IELs) are not exclusively T cells as thought before and hence the intestinal epithelium also seems to be a site where B cells are recruited. In rainbow trout, oral vaccination with an alginate encapsulated DNA vaccine against IPNV resulted in increased IgM+ and IgT+ B cell populations, an indication that both B cells are important for mucosal responses [44]. However, Zhang et al. [34,35] reported that IgT is the main immunoglobulin responsible for mucosal immunity. It has to be noted that the aforementioned studies [35,44], differed in the pathogen examined (parasite *vs* virus) and the timing of the responses measured (late *vs* early). In addition to the already assigned mucosal role of IgT, its involvement in systemic responses cannot be neglected as observed in trout spleen [45]. Accordingly, Castro et al. [45] has described intestinal IgM+ and IgT+ cells in trout as B cells, even though immunocytochemical observations do not provide any evidence on the presence of plasma cells. In a much earlier study on common carp, staining (mAb WCI12) of the gut IELs for membrane and cytoplasmic IgM indicated that the majority of Ig+ IELs were small plasma cells; having a rim of Ig+ cytoplasm and a minor amount of membrane Ig [46]. These findings in trout and carp may be pointing to the fact that teleost gut has a limited number of classical plasma cells and that they are not easily detectable in the mucosal tissues. Further investigations are essential for understanding the existence and role of IgZ2 or IgT plasma cells in the gut of teleosts.

A variety of Ig genes is present in fishes. The evolutionary origin of the mucosa-associated IgT is yet to be clarified, and its appearance in some lineages of bony fishes could be due to selection pressures arising from the necessity to protect the mucosal surfaces [47]. Further, IgT/Z shares many functional similarities with mammalian IgA [22]. Even if IgT/IgZ cannot serve as IgA equivalent in teleosts, we cannot neglect the “power” of alternative splicing of pre-mRNA in fish, recently summarized by Maisey and Imarai [48] and Quiniou et al. [49]. Such splicing may also be responsible for differences in IgM heavy chains that can result in mucosal and