MODULATION OF NONSPECIFIC DEFENCE MECHANISMS AND PROTECTION AGAINST DISEASES IN FISH

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Abstract

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The use of immunomodulators in fish culture offers a wide range of attractive methods for inducing or modulating protection against diseases. Several promising synthetic drugs and biological response modifiers stimulate or modulate the nonspecific defence mechanisms and specific cellular and humoral immune responses in fish. This article reviews the literature on the influence of biostimulants on the cellular and humoral defence mechanisms in fish, and present the possibility to application of immunostimulants for control of infectious diseases in aquaculture.

Fish, immunostimulants, defence mechanisms

Protection of fish against bacterial diseases by specific vaccines has been an important method for many years in aquaculture. However, defence mechanisms in the lower vertebrates are somewhat different from those in mammals, and some immunization techniques when actually applied to hatchery conditions are not as effective as they should be. Therefore, research is concentrating on how to improve the potency and efficacy of the antigens and how to optimally activate the nonspecific defence mechanisms and specific cellular and humoral immune responses. When a fish initially encounters a pathogen, the nonspecific defence mechanisms are more important than the specific immune response, as the latter requires a long time for antibody build-up and specific cellular activation. In general, fish have short life spans and most live in cool water environments which slows development of the specific immune response.

Nonspecific defence barriers and mechanisms

The nonspecific defence barriers already in place include physical epithelial shield of the scales, skin and the mucus. If an infectious agent is entrapped by the mucopolysaccharide complexes of the mucus, it may be scuffed from the fish or held to be digested by the mucus lytic enzymes. In most cases, the pathogenic microorganisms are destroyed by digestive and lytic enzymes. Inflammation may result at a tissue-damaged site, resulting in the migration of leukocytes to wound areas and the elevation of serum component concentrations, including C-reactive protein, transferrin, lysozyme, ceruloplasmine and complement components.

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Phagocytes and phagocytosis are the main tools of the nonspecific immune response to pathogenic factors such as viruses, bacteria and parasites. The effector functions of phagocytes are considerable, but most important is their ability to kill pathogens. Both PMN and MN phagocytic cells arrive at a site of inflammatory reaction by chemotaxis. They may then attack microorganisms via their nonspecific cell surface receptors, or if the microorganism is opsonized with the fragment of the complement component (C3b) through activation of the complement system, attachment will be through the cell surface receptors for C3b. If the membrane does not become activated by the attached infectious agent, this is engulfed into a phagosome by pseudopods which extend around it. Once inside, lysosomes fuse with the phagosome forming a phagolysosome and the infectious agent is killed by a battery of microbicidal mechanisms.

Viral agents may induce the production of interferons by viral-infected cells, stimulate other cells to activate intracellular endonucleases that will destroy viral DNA and RNA and will shut down physiological pathways that viruses use for replication. This phenomenon is mediated by a population of nonspecific cytotoxic (NC) cells. Similar to natural killer (NK) cells, the NC cells are non-T, non-B cells which are not phagocytic. Compared to NK cells, NC cells produce very high levels of *in vitro* cytotoxicity against a wide variety of mouse and human target cells.

Specific immune response

Antibody is the primary result of the specific immune response, and unique in the fish physiology because these immunoglobulin molecules are specifically directed against individual antigens. The common Ig in fish is a tetrameric form of antibody and is usually designated as IgM. By contrast, the dimeric form is common (IgG) and efficient in mammals with a pentameric form (IgM) being a molecule produced early in infection or immunization. The antibody is a recognition molecule and as such has several function :

- it can complex with antigens and initiate other serum proteins to begin a complement cascade in lysing invasive pathogen,
- it can be stationary or attached to cells or act as a communicator to direct circulating cytotoxic cells to recognise and attack pathogenic agents.

The initiation of the specific immune response is controlled by MN phagocytic cells (antigen presenting cells - APC) involved in particle or antigen uptake. The information concerning what to make antibody against is transferred to the B cells. Complex receptors on the cells, such as the major histocompatibility complexes (MHCs), that guide the selection of the antibody, assisted by messenger chemical molecules, cytokines and interleukins, that are involved in the expression and control antibody secretion. Many of these messenger molecules are produced by subpopulation of T-lymphocytes, cytotoxic cells and phagocytes. Indeed, many of these cells, armed by information on what to attack physically participate in destruction of the pathogens. The specific immune response, in contrast to nonspecific defence mechanisms, can hold memory, and when the animal meet the pathogen for a second time, antibody is more rapidly produced and in a greater quantity.

Modulation of cellular and humoral immunity

The immune status of fish held in hatcheries, farms and aquaculture netpens is negatively effected by many factors: including polyetiological stress, environment pollution and treatments with chemotherapeutics (Anderson 1990; Dunier et al. 1991; Dunier and Siwicki 1993; Siwicki et al. 1997; Studnicka et al. 1997; Van Muisvinkel et al. 1985; Wishkowsky et al. 1987). The effects of these factors may impair the protective mechanisms of the fish and together with the accumulation of various flora in aquatic environment, conditions become favourable to the occurrence of infectious diseases, frequently with a mixed etiology. Therefore, it is of great importance to study the early detection of immune deficiencies and the stimulation or modulation of nonspecific cellular and humoral immunity. The use of immunomodulators and adjuvants in fish culture offers a wide range of attractive methods for inducing and modulating protection against diseases. But the exact mechanism of action by immunostimulant and/or immunomodulator on the biophysical pathways is often unknown. In general, immunostimulants and/or immunomodulators comprise a group of synthetic and biological compounds that enhance the cell-mediated and humoral-mediated immunity in animals. Some of them only stimulate the defence mechanism, others are able to restore or modulate immunity after suppression induced by xenobiotics. The stimulation of the defence mechanism may be particularly important for fish that are raised in or released to environments where the species or serotypes of pathogens are unknown and immunization by specific vaccines may be futile.

Several promising adjuvants, drugs and biological modifiers have been tested *in vitro* and *in vivo* in fish. A summary of biostimulants and/or biomodulators used for activation or restoration the nonspecific defence mechanisms in fish and specific immune responses are presented in Table 1.

Table 1.
The influence of biostimulants and /or biomodulators on the cellular and humoral defence mechanisms in fish - summary from published scientific papers.

Biomodulator	Results	References
Bacille Calmette Guerin (BCG)	increased	Grayson et al. 1987
Boviglobin	increased	Siwicki et al. 1996
Candida utilis (yeast)	increased	Siwicki et al. 1994
Ceromangan	increased	Siwicki et al. 1996
CFA (modified)	increased	Olivier et al. 1985
Chitosan	increased modulated	Siwicki et al. 1994 Anderson, Siwicki 1994
Evetsel	increased	Siwicki et al. 1994
FinnStim	increased	Siwicki et al. 1994
FK-565	increased	Kitao et al. 1986
Glucan 1,3	increased	Yano et al. 1989
M-Glucan	increased	Robertsen et al. 1990
ß-glucan	increased	Nikl et al. 1991 Siwicki et al. 1994
HMB	increased	Siwicki et al. 1998
ISK	increased	Jeney and Anderson 1993
Lysozyme dimer (KLP-602)	increased modulated modulated modulated	Morand et al. 1998 Klein et al. 1997 Studnicka et al. 1998 Siwicki et al. 1998
Saccharomyces cerevisiae	increased	Siwicki et al. 1994
Watasenia scintillans (heat extract)	increased	Siwicki et al. 1996

Complete Freund's adjuvant (CFA) was one of the first immunostimulants used in animals and humans to elevate the specific immune response, and it has also successfully been used in conjunction with the injection of the bacterins (Olivier et al. 1985). Other immunostimulants and biological response modifiers that have been used in fish research include synthetic levamisole (Siwicki and Anderson 1990), bacterial lipopolysaccharides and glucans (Yano et al. 1989). Vaccines have been adsorbed to inert particles, such as bentonite and latex beads to carry the immunogens in attempts to maximise *in vivo* uptake for bath immunization and to facilitate *in vitro* phagocytosis. Each substance presents special problems in timing and methods of administration by injection, immersion, orally or flush treatments, and dosage adjustments for size and fish species. An additional consideration is that the nonspecific defence mechanisms and specific cellular and humoral responses are highly variable among individuals and statistical validation requires appropriate sample numbers and carefully controlled experiments. The preliminary studies, assaying the influence of the biostimulants can be done *in vitro* (Siwicki et al. 1990).

The influence of immunomodulators on the fish cellular defence mechanisms can be followed by taking samples of nonlethal blood and haematopoietic organs and observing changes in leukocyte numbers, activity and functions. Macrophages/monocytes, neutrophils and other phagocytic cells increase the enzyme mobilisation, oxidative radical production and phagocytic activities. The subpopulations of T lymphocytes increase the proliferative responses and cytotoxic activity, as well as increase the proliferative response of B lymphocytes and level of antibody secreting cells. The effect of stimulants on fish humoral defence mechanisms can be followed in blood samples analysed for changes serum or plasma total Ig and specific antibody levels, lysozyme and ceruloplasmin activity and cytokines levels (Anderson 1992; Anderson et al. 1992; Siwicki and Anderson 1993; Siwicki et al. 1993).

Immunostimulants may be used in patterns similar to those of chemotherapeutics or chemicals and in combination with vaccines. The fish could be prepared for a predicted event, such as seasonal exposure to pathogens or handling stress, by a treatment prior to the event. Many environmental and physiological variables will influence experiments and protocol for the use of immunostimulants in fish, including timing, dosage requirements, environmental temperature, stability of each component, the characteristics of the vaccine and species of fish. The first consideration is whether the substance is to be used alone as a single treatment or whether it will be used in conjunction with an immunization program.

Several fish food manufactures now offer diets supplemented with biostimulants for use in aquaculture. Currently recommended schedules for feeding some immunostimulants call for administration every day for 1 to 2 weeks (Onarheim 1992). Many questions remain concerning the regimens and use of immunostimulants, particularly as to routes of administration, appropriate dosages, time and length of application, and influence on the environment.

Modulace nespecifických obranných mechanismů a ochrana před nemocemi u ryb

Užití imunomodulátorů v rybářství nabízí široké spektrum atraktivních metod pro indukci nebo modulaci ochrany před nemocemi. Některá nadějná syntetická léčiva a biologické odpovědi modifikují stimulačně nebo modulačně nespecifické obranné mechanismy a specifické buněčné nebo humorální imunologické odpovědi ryb. Příspěvek obsahuje přehled literárních údajů o vlivu biostimulantů na buněčné a humorální obrané mechanismy ryb a předkládá možnost aplikace imunostimulačních látek pro kontrolu infekčních nemocí v akvakultuře.

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