# Trained immunity: a sustainable disease management strategy in aquaculture

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Diseases and their management are of major concern in aquaculture. A disease occurs when the intricate relation between host–pathogen–environment is affected. Earlier, the emphasis was only on increased production from this sector. However, presently along with production, emphasis is also given towards responsible and sustainable aquaculture practices. This limits the use of antibiotics and other chemotherapeutic agents in aquaculture. Disease management in aquaculture now mainly relies on prophylaxis such as the application of probiotic microorganisms, water quality management, vaccines and immunostimulants. Strengthening the immune system is pivotal in aquaculture as most fish pathogens are opportunistic in nature. The application of vaccines is one of the best prophylactic measures in aquaculture that stimulates acquired immune memory response. However, complications in vaccine development, practical difficulties in vaccine delivery and economic feasibility limit its application in aquaculture. Moreover, the major share of aquaculture is from the invertebrate culture that lacks the acquired immune system.

The immune system acts are based on four processes: recognize the pathogen, respond, remove and remember it to achieve homeostasis. These four Rs are balanced by the two arms of the immune system – the innate and adaptive immunity. The major difference between innate and adaptive immunity is in immunological memory. However, recent studies on different organisms demonstrated the existence of immune memory mediated through the cells of the innate immune system. There are several interesting studies on the existence of innate immunological memory in invertebrates and vertebrates, including humans[1]. In higher vertebrates it is known as priming of the immune cells, induction of innate memory, memory-induced enhanced responsiveness, etc. Netea *et al.*[1] proposed a new term 'trained immunity' for describing the innate immune memory responses.

## Trained immunity – an innate immune memory programme

A primary infection or immunization induces the innate immune system, providing protection against a subsequent secondary infection in a T and B lymphocyte-independent manner. It may be less specific than adaptive immune response, but still confers increased resistance upon reinfection of the host. Innate cells such as NK cells and macrophages are the key players of this mechanism (improved pathogen recognition and inflammatory response). The training of immune cells may go in either direction of homeostasis (Figure 1). Mostly it enhances immune responses, but rarely it may cause a decrease in the response of immune cells to antigens, which is known as 'immunological tolerance'[2]. This decrease in the response of immune cells to the antigen can be minimized by the use of proper stimulus (effectors) with appropriate dosage and time. These effectors are the infectious or non-infectious agents, including bacterial, fungal cells, viruses, parasites, and their components (e.g. $\beta$-glucan, chitin, BCG vaccine or LPS). This signalling triggers the entire training along with the direction of the training process. Once the stimulation is completed, the immune cells will be initiated in a T and B cell-independent process, followed by epigenetic changes leading to the altered metabolic programming.

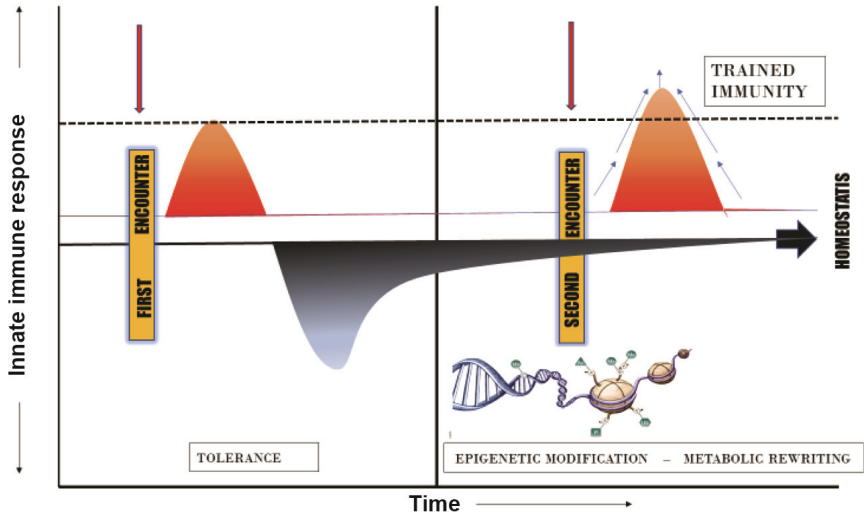


**Figure 1.** Trained and tolerance phases of innate immune cells. Two phases of immune cells (trained and tolerance) upon first and second stimulation (red solid arrows) are depicted. The elevation and demotion from the homeostasis line show the response of immune cells towards the priming process – the elevated portion above the dotted line indicates upregulation of the trained immune cells as result of epigenetic and metabolic reprogramming.

## Epigenetics

This refers to processes that result in heritable alterations in gene activity without manipulating the underlying DNA sequence[3]. DNA methylation, histone modifications, chromatin remodelling and noncoding RNA activities are the major mechanisms involved in epigenetics. During these processes, up- and down-regulatory effects may occur within the memory induction training. In DNA methylation, addition of methyl(-$CH_3$) groups to the cytosine ring forms 5-methylcytosine. Griffith and Mahler[4] proposed that in DNA methylation the whole addition process is under the action of the enzymatic machinery, including the maintenance of methyl transferase (DNMT1) and *de novo* methyltransferases DNMT3A/3B. Activation and repression of genes depend on the methylation at CpG islands in the promoter region. Histone modification

is a post-translational process which occurs by methylation, phosphorylation, acetylation, ubiquitylation and sumoylation. Here methylation is catalysed by histone methylase (HMTs) and histone demethylase (HDM), whereas acetylation is under the control of histone acetylase (HATs) and histone deacetylase (HDACs). The former enables 'relaxed' chromatin state, thereby enabling the attachment of transcription factor (TF). In histone methylation, H3K4, H3K36 and H3K79 mark active transcription, whereas H3K9, H3K27 and H4K20 are considered to be associated with silenced chromatins. This complex dynamic interchanging process followed by the remodelling of chromatin also acts as an epigenetic mark in this memory induction process.

## Metabolic rewriting

The involvement of various pathways such as glycolysis, tricarboxylic acid (TCA) cycle, fatty acid oxidation, fatty acid synthesis, pentose phosphate pathway and amino acid synthesis has significant impact on immune cell reprogramming. The ultimate aim of the cell is to generate and gather energy. The biochemical demand of each immune cell is intrinsically linked to its function. The requirement and pathway of acquiring energy change when a resting cell gets immune-activated. The resting immune cells utilize energetically efficient processes such as TCA, linked to the generation of ATP via oxidative phosphorylation. Whereas activated cells prefer glycolysis to generate sufficient energy storage to support survival and cellular growth. Along with this shift, glutaminolysis and intermediators of the Kreb cycle and cholesterol pathway have a role in trained immunity as they provide metabolites, and thus several metabolic enzymes as cofactors of epigenetic reprogramming enzymes. The accumulation of fumarate in the TCA cycle induces epigenetic reprogramming by inhibiting the enzyme which sheds away the methyl groups from the histones (KDM5 histone demethylases). This leads to an easier transcription in the second encounter by keeping the chromatin open and providing a platform for attachment of the TF. Interchanging of fluvastatin with mevalonate in the cholesterol synthesis pathway shapes the role of cholesterol in the innate memory training process, especially the trimethylation at H3K4 position, which is a downregulation activity. Most importantly, the TCA cycle itself acts as an acetyl donor to the H3K27Ac process for the opening of the chromatin. Rewriting this metabolic profile is necessary as the Krebs cycle itself inhibits some of the epigenetic programmes.

## Trained immunity in aquaculture

Several studies on the potential of trained immunity in aquaculture mostly highlight the best aquaculture practices through proper aquatic animal health management, based on advanced biotechnology and genetics. The trained immunity can be connected with nutritional reprogramming, in which any sort of nutritional exposure during the 'critical window' can permanently change many physiological processes[5]. Research on nutritional programming in fishes has been initiated recently[6,7]. Here, the critical window is generally indicated as the critical developmental periods such as larval stages or spawning season (brood stock). Since shrimps are devoid of the acquired immune system, training of this innate immune response is a promising strategy for disease control in shrimp aquaculture[8]. Though immunostimulants have been used in aquaculture for years, the actual mechanism (trained immunity) through which the culture is protected has been deciphered recently. Through training, the innate immunity shrimp displayed an increase in resistance against pathogens within the generation and even across generations[9]. Trained immunity also caused changes in vaccinology, such as the development of trained immunity-based vaccines defined as vaccine formulations that induce training of innate immune cells. Many of the currently used anti-infectious vaccines, immunostimulants and even vaccine adjuvants come under this category. Trained immunity brought a paradigm shift in our understanding of the immune system in fishes and shell fishes. This concept is now widely utilized for the activation of the immune system of cultured species through which we can curb disease outbreaks in aquaculture and subsequently limit the use of chemotherapeutics.


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