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Introduction

Shrimp immunity and disease control

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Abstract

The sustainability and development of shrimp aquaculture are largely at stake as significant ecological and pathological problems are increasing in the vast majority of the shrimp producing countries. Prevention and control of diseases are now the priority for the durability of this industry. Within the past decade, intensification of the shrimp production, based on progress in zootechnology, has increased but with little corresponding increase in scientific knowledge of shrimp physiology. Within this field, shrimp immunology is a key element in establishing strategies for the control of diseases in shrimp aquaculture. Research needs to be directed towards the development of assays to evaluate and monitor the immune state of shrimp. The establishment of regular immune checkups will permit the detection of shrimp immunodeficiencies but also to help monitor and improve environment quality. For this, immune effectors must be first identified and characterised. In the end, however, the assumption may be made that the sustainability of aquaculture will depend on the selection of disease-resistant shrimp, i.e. to develop research in immunology and genetics at the same time.

The development of strategies for prophylaxis and control of shrimp diseases could be aided by the establishment of a collaborative network to contribute to progress in basic knowledge of penaeid immunity. However, to improve efficiency, it appears essential also to open this network to complementary research areas related to shrimp pathology, physiology, genetics and environment.

Keywords: Penaeid shrimp; Immunity; Prophylaxis; Selection; Disease resistance

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1. Introduction

Shrimp aquaculture started in the 1970s as an industrial activity and developed rapidly with a huge increase in the number of hatcheries and farms. Shrimp farming provides roughly 30% of the shrimp supplied to the world market. The activity concerns tropical countries in South East Asia, Central and South America and the total annual production reaches about 712 000 metric tonnes (Rosenberry, 1996).

The industry saw major growth during the 1980s, but now, production is regularly and seriously affected by problems linked to environment degradation and to infectious and non-infectious diseases. This situation has grown worse with an intensification of shrimp farming based on progress in zootechnology but with a lack of knowledge of penaeid physiology and often with little consideration of ecological aspects. The causative agents of infectious diseases in shrimp are mainly viruses and bacteria belonging to Vibrionacea. These pathologies particularly hamper larval production and lead to profitability problems due to stock mortalities. They also lead to the overfishing of wild shrimp larvae and an overexploitation of broodstock. Moreover, the local environment can be contaminated by the discharge of wastewater containing antibiotics, which can foster the development of drug-resistant bacteria. Finally, the practice of shrimp transfers at national or international levels has contributed to the spread of diseases, which are now an enzootic. Non-infectious diseases are often suspected to occur because of environmental degradation exacerbated by inappropriate management practices.

Consequently, the control of disease became a priority at the world level if shrimp production is to be ecologically and economically sustainable. To a greater extent, the durability of the production is dependent on the equilibrium between — (i) the environment quality, (ii) the prevention of diseases by diagnosis and epidemiological surveys of the pathogens, and (iii) the health status of the shrimp. Finally, the shrimp aquaculture is also dependent on the selection of animals resistant to diseases. Therefore, the prevention and the control of shrimp diseases need an integrated approach in which knowledge of shrimp immunity must be improved, consideration given to other research areas related to pathology and shrimp physiology, and in close connection to research in genetics. In this spirit, a collaborative research project “Shrimp Immunity and Disease Control” (SI & DC) has been established and is supported (1997–2000) by the European Commission (DG XII) as a Concerted Action in the programme International Cooperation with Developing Countries, INCO-DC (project no. IC18CT970209).

2. Expected outcomes from the SI & DC project

For the prevention of shrimp diseases, just as with human and other areas of animal health, it is crucial to develop quantitative assays for the evaluation and the monitoring of the immune state of shrimp. The establishment of regular health checks would permit the detection of shrimp immunodeficiencies and, consequently, reduce disease vulnerabilty, but at the same time, it could also contribute to the control and improvement of environment quality (Bachère et al., 1995).

In the long term, as with plant and animal genetics (Strittmatter and Wegener, 1993; Müller and Brem, 1994), one can assume that the most effective way to ensure continued shrimp production will depend on the selection of disease-resistant shrimp, and that means developing parallel research in immunology and genetics. The measurement of the defence reactions as well as the characterisation of immune genes could be exploited in quantitative genetic selection as traits for increased resistance to diseases (Bachère et al., 1995). Genetic transformation may constitute another promising strategy for the obtainment of resistant strains by modifying the expression of immune encoding genes or by expressing foreign genes, characterised in other species, to confer new traits of resistance into the shrimps (Mialhe et al., 1995, 1997; Bachère et al., 1997).

3. Scientific programme of the SI & DC project

The programme concerns the main aquacultured shrimp species, *Penaeus monodon* (South East Asia), *P. vannamei* and *P. stylirostris* (Latin America and Pacific) but also *P. japonicus, P. semisulcatus* (Europe, Japan) and *P. paulensis* (Brazil) as well as the freshwater crayfish *Pacifastacus leniusculus*.

The scientific programme of the SI & DC project aims at the characterisation of defence effectors in shrimp, considering knowledge and experience acquired in other arthropods. The innate defence mechanisms are based on both cellular and humoral components of the circulatory system, which interplay for detecting and for eliminating foreign and potentially harmful microorganisms and parasites. The immune response in arthropods can be subdivided into different phases: (i) an immediate and inductive stage corresponding to the recognition of the non-self factors, and to the initiation of immune reactivity, (ii) a cellular and synthesis stage of effectors, (iii) a final humoral and cellular stage of recovery (Ratcliffe, 1993).

3.1. Specific actions

Within the overall framework of the SI & DC project, specific actions deal with the functional, biochemical, antigenic and genetic characterisation of effectors involved and expressed in response to pathological injuries or to stressful conditions (Fig. 1).

3.1.1. Non-self recognition — action 1

The first immune process is the recognition of invading microorganisms, which is mediated by the haemocytes and by plasmatic proteins (as reviewed by Vargas-Albores and Yepiz-Plascencia, this issue; Marques and Barracco, this issue). There is little information about the molecular mechanisms that mediate recognition; however, in crustaceans, several types of modulator proteins have been described that recognise cell wall components of microorganisms.

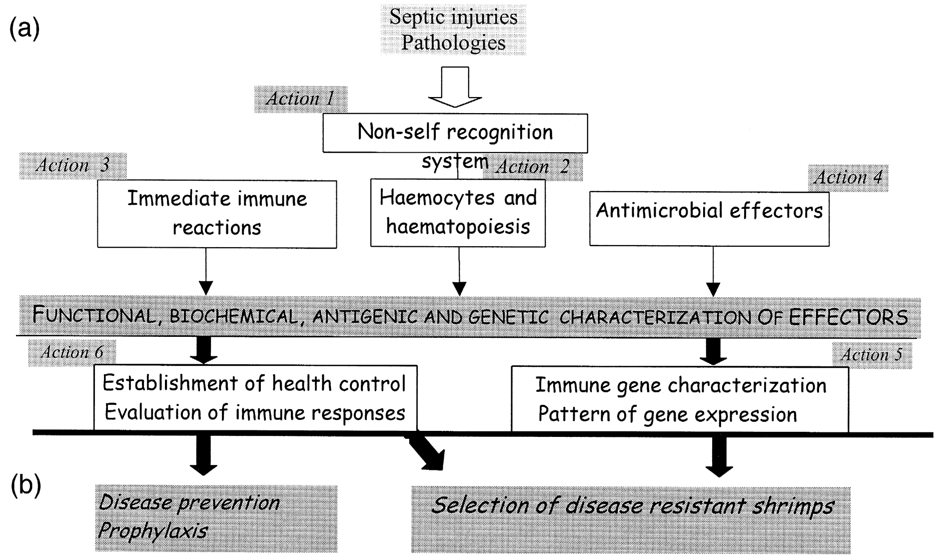

Fig. 1. Specific actions (a) of the SI&DC project and expected applications (b) in the prevention and the control of disease in penaeid shrimps.

Briefly, in the freshwater crayfish *P. leniusculus*, a β-1,3-glucan-binding protein has been characterised and cloned, and a haemocyte receptor has been partially characterised that binds the plasmatic glucan-binding protein after the latter has reacted with β-1,3-glucan (Duvic and Söderhäll, 1992). In the shrimp *P. californiensis*, a protein that responds to β-1,3-glucan has been purified and characterised (Vargas-Albores et al., 1996).

Recently, a peptidoglycan recognition protein was purified from the haemolymph of *Bombyx mori*, capable of activating the prophenoloxidase (proPO) cascade (Yoshida et al., 1996).

Various lipopolysaccharide-binding proteins (LPS-BP) have been characterised in different arthropods: i.e. in the insect, *Periplaneta americana*, a lectin entraps bacteria within the haemolymph and participates in tissue regeneration (Jomori and Natori, 1992; Natori and Kubo, 1996); lipophorin detoxifies LPS from *B. mori* haemolymph (Kato et al., 1994); hemolin, a circulating protein with immunoglobulin-like domains characterised in the blood of some lepidopterans, can bind to bacteria and possibly functions as an opsonin (Lanz-Mendoza et al., 1996); in the Chelicerate, *Tachypleus tridentatus*, various proteins with LPS-binding properties have been characterised as lectins such as tachylectins, serine proteinases such as factor C, which induces coagulation cascade (for review, see Iwanaga et al., 1998). In shrimp and crayfish plasma, LPS-BP that are agglutinins have also been purified but their exact role remains to be determined (Kopacek et al., 1993a, b; Vargas-Albores et al., 1993a, b).

Considering the importance of bacterial diseases in shrimps, a special attention must be devoted to the study of the molecules or mechanisms involved in the recognition of the bacteria belonging to the Vibrionacea group.

3.1.2. Circulating haemocytes and haematopoieitic cells — action 2 (reviewed by Johansson et al., this issue)

The recognition molecules may interact with and activate the haemocytes, which play an important and central role in host defence. In crustaceans, according to numerous works dealing with the identification of haemocyte cell types, a classification scheme has been commonly adopted with three types of circulating haemocytes: hyaline, semigranular and granular cells (Bauchau, 1981; Martin and Graves, 1985). Based on morphological and cytochemical characterisations, some functions and involvement in different defence reactions have been attributed to the different cell types, i.e. the involvement of the hyaline cells with coagulation (Omori et al., 1989), of the granular and semigranular cells with phagocytosis (Gargioni and Barracco, 1998) and with the proPO system (Johansson and Söderhäll, 1985). Flow cytometry analyses of the haemocyte populations of the shrimp *P. japonicus* also revealed three populations according to the cell size and to cell granularity and structure (Sequeira et al., 1995). However, divergent results with hemogram composition and determination of cell populations are still reported by different authors. Such contradictory results are particularly evident when analyses are made on the behaviour and the involvement of the haemocyte populations in the response to immune stimulation. Recently, characterisations of the shrimp haemocytes by means of the production of specific monoclonal antibodies were undertaken leading to the identification of proteins specifically expressed in different cell types (Rodriguez et al., 1995). New approaches associating flow cytometric analyses and molecular markers specific for haemocytic immune proteins will permit to better determine the role and function of the different cell populations and to establish the cell lineage, questions which largely remain debated in crustaceans.

3.1.3. Immediate defence systems — action 3

The non-self-recognition factors activate several immediate defence systems mediated by the haemocytes. The proPO-activating system is indubitably one of the best-studied immune systems in crustaceans with numerous published works on the crayfish (reviewed in Sritunyalucksana and Söderhäll, this issue). Numerous components associated with this immediate defence process, which leads to the reaction of melanisation, have been characterised and some functions in the proPO-activating system can be now proposed for most of them (Söderhäll and Cerenius, 1998); the activation (triggering) of haemolymph coagulation is closely related to that of the proPO system. Coagulation is a rapid and powerful reaction in crustaceans. The clotting process has been studied in some details in the crayfish. Preventing blood loss upon wounding is also an important defence reaction by participating in the engulfing of foreign invading organisms (Kopacek et al., 1993a, b). In shrimps, some components of these defence reactions have been identified or have begun to be characterised, i.e. proPO-activating system (Vargas-Albores et al., 1993a, b; Perazzolo and Barracco, 1997), a plasmatic clotting factor and α2-macroglobulin characterised by means of specific monoclonal antibodies (Bachère et al., 1995; Rodriguez et al., 1995). The defence reactions also include the haemocytic process of encapsulation, phagocytosis and the microbicidal mechanism based on the production of cytotoxic reactive oxygen intermediates demonstrated in crab (Bell and Smith, 1993) and shrimps (Song and Hsieh, 1994; Bachère et al., 1995).

The role and real function of these immediate defence reactions have to be analysed in regard to pathological injuries that means first to fully characterise their components in shrimps.

3.1.4. Antimicrobial proteins or peptides — action 4

The innate immune response of arthropods also relies upon the production of antimicrobial peptides that are active against a large range of pathogens. In the horseshoe crab (Chelicerata), the haemocytes, upon endotoxin activation, release by exocytosis the contents of two types of granules including, on the one hand, the clotting factors essential for haemolymph coagulation, proteases, tachylectins, and on the other hand, antimicrobial peptides, tachyplesins, big defensin, tachycitin and tachystatins, which present agglutinating activity (reviewed in Iwanaga et al., 1998). In insects, the synthesis of antimicrobial peptides induced upon injury is the hallmark of the immune response of higher insect orders. A septic injury induces the rapid and transient transcription of several genes encoding potent antibacterial and antifungal peptides that are released into the blood where they act to destroy the invading microorganisms. The activation cascades that control the expression of the antimicrobial peptide genes in higher insects show striking structural and functional similarities with activation cascades involved in cytokine-induced expression of acute phase proteins in mammals (Hultmark, 1993; Hoffmann and Reichhart, 1997).

The production of antimicrobial peptides is in fact widespread in the living kingdom, from bacteria to plants, and from vertebrates to invertebrates (for review, see Bachère et al., this issue). Surprisingly, in crustaceans, until now, the innate defence reaction involving the synthesis of antimicrobial peptides has been poorly studied. To date, constitutive haemocytic proteins have been isolated in the crab *Carcinus maenas*, and a 6.5 kDa antimicrobial peptide has been partially characterised (Schnapp et al., 1996). In the penaeid shrimp, *P. vannamei*, three antimicrobial peptides have been purified from the haemocytes and the plasma. They have been fully characterised and their cDNA cloned. According to their biochemical and structural features, the three peptides could not be associated with other peptide families hitherto described, and they were named penaeidins, after the genus *Penaeus* (Destoumieux et al., 1997). Research is being carried out into the role of the penaeidins in the immune response against pathogens as well as with the characterisation of other peptides in shrimp.

3.1.5. Immune effector encoding genes and gene expression regulation — action 5

Besides the biochemical or antigenic characterisation and the determination of the biological activities of the immune effectors, one may focus on their cloning and on the characterisation of their encoding genes. The analyses of the pattern of expression of the effector encoding genes during the response to infections or to immune stimulations will lead to a better understanding of their involvement in defence reactions against some specific harmful pathogens. In this objective, the determination of the mechanisms that control and regulate the expression of the effectors is of prime importance.

3.1.6. Evaluation of immune responses and health management — action 6

Compared to that of mammalian breeding, understanding of criteria for evaluating the health state of shrimp, and of invertebrates in general, are practically non-existent. The establishment of health monitoring in shrimp needs firstly, to develop and to standardise quantitative assays for measuring the immune reactions and the expression of effectors. These assays must be miniaturised for simultaneous individual analyses of different effectors. Moreover, they need to be adapted for analyses of individual variability considering physiological parameters, such as the moult cycle, age, sex or breeding stage, and under controlled physico-chemical or environmental parameters. The effects of organic or chemical pollutants, antibiotics used in aquaculture, should be studied and the real effect of immunostimulants, which have been until now used empirically, could be examined.

The immune responses need to be analysed under stress conditions and pathological injuries in cultured shrimp for the validation of health monitoring at population level.

4. Objectives of the SI&DC concerted action

To ensure successful shrimp culture by preventing and controlling disease, there is an urgent need to organise and develop research on penaeid shrimp. New methodologies and new concepts for shrimp aquaculture must be established and then developed with and for the producers. Considering the importance of shrimp aquaculture and the limitation of this industry due to pathological problems, one can notice a lack of specialists in the fields of pathology, immunology and genetics of penaeids, particularly in the producing countries. The crisis situation can be explained by the fact that this industry developed rapidly with very little scientific knowledge to support the new farming techniques. The training of local scientists specialised in shrimp pathology, immunology and genetics is, thus, of prime importance.

In this attempt, the SI&DC Concerted Action, supported by the DGXII (European Commission) (Sept. 1997–Sept. 2000), is intended to increase basic knowledge of penaeid immunity by creating an open collaborative network of research laboratories involved in shrimp immunity and in other invertebrate groups. In this relatively new area, the communication and the collaboration must also be improved between ongoing research projects in different parts of the world. The greatest benefits for joining countries, beyond the avoidance of duplication, would lie in cooperation rather than competition, and in exchanges of information, hence, improving efficacy, that is the definition of a concerted action (Loukopoulos, D., personal communication). Through the SI&DC project, it is expected to exchange results and reagents, to transfer and standardise technologies and methodologies, but it is also expected to improve the training of young scientists in shrimp producing countries.

Finally, the SI&DC project might initiate new ideas and new supported projects. Moreover, in the framework of the SI&DC Concerted Action, one of the objectives is to transfer and to exchange information and results with other areas, not only pathology and genetics, but also with nutrition, reproduction, environment and ecotoxicology. These research domains must be closely related to immunity for the development of new approaches dealing with the prevention and the control of diseases in shrimp aquaculture.

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