Fish & Shellfish Immunology 35 (2013) 979–987

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Fish cell cultures as *in vitro* models of inflammatory responses elicited by immunostimulants. Expression of regulatory genes of the innate immune response

C. Fierro-Castro [1], L. Barrioluengo, P. López-Fierro, B.E. Razquin, A.J. Villena*

Departament of Molecular Biology (Cell Biology Unit), Facultad de Ciencias Biológicas y Ambientales, Universidad de León, 24071 León, Spain

ARTICLE INFO

Article history:
Received 19 May 2013
Received in revised form
22 June 2013
Accepted 9 July 2013
Available online 18 July 2013

Keywords:
Immunostimulants
Innate immunity
Gene expression
Lympho-haematopoietic stroma
Oncorhynchus mykiss

ABSTRACT

We report the differential expression of various genes related to the regulation of the innate immune responses, including pro-inflammatory (IL-1β1, IL-8, TNF-α1, TNF-α2) and immune-suppressing (IL-10) cytokines, interferon-induced Mx-1 protein, enzymes regulating nitric oxide (inducible nitric oxide synthase, arginase-2) and eicosanoid (COX-2) production, and Toll-like pathogen pattern-recognition receptors TLR-3, TLR-5 and TLR-9, in two lympho-haematopoietic stromal cell lines derived from the spleen (trout splenic stroma, TSS) and the pronephros (trout pronephric stroma-2, TPS-2) of rainbow trout (*Oncorhynchus mykiss*), as well as in primary cultures of rainbow trout head kidney macrophages, after their exposure to the well-known immunostimulants LPS, levamisole and poly I:C.

Although there were differences in the responses between the two stromal cell lines, using reverse transcription followed by real time polymerase chain reaction (RT-qPCR) we demonstrated that exposure to the immunostimulants, particularly poly I:C and LPS, resulted in significant changes in the expression of the immunoregulatory genes in the two stromal cell lines in many cases their responses resembling in fold change magnitudes and in response profiles to those observed in the primary macrophage cultures. Exposure to poly I:C and, with lower fold change values, to LPS produced upregulation of the pro- (IL-1β, IL-8, TNF-α) and anti-inflammatory (IL-10) cytokine genes, as well as of the Mx-1 gene. Furthermore, the immunostimulation elicited the upregulation of COX-2, iNOS and arginase-2 genes in the cell lines. Likewise, the TSS and TPS-2 cell lines significantly upregulated the expression of TLR-3, TLR-5 and TLR-9 genes after exposure to the immunostimulants, thus explaining the ability of the stromal cells to recognise and respond to the immunostimulants.

Such results give support to an important role of lympho-haematopoietic stromal cells in the development and control of pro-inflammatory responses in fish. The upregulation of genes of pro-inflammatory cytokines and of mediators of the innate immune responses correlates well with the previously demonstrated functional capacities, including phagocytosis, microbicidal activity and NO production, exhibited by the TSS and TPS-2 stromal cell lines when exposed to the same immunostimulants. On the other hand, the expression of immunosuppressing genes (IL-10, COX-2 and arginase-2) demonstrate that the lympho-haematopoietic stromal cells are also able to contribute to the control of inflammatory responses.

This study reinforce the possibility of using histotypic cell cultures, as those formed by the TSS and TPS-2 cell lines, formed by heterogeneous cell populations that partially replicates the cell–cell and cell-extracellular matrix interactions, to develop cost-effective and repetitive *in vitro* systems for the screening of immunostimulant candidates for aquaculture, as they are able to replicate *in vitro* immune regulatory networks occurring *in vivo*.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Immunostimulants, which primarily activate non-specific cellular mechanisms mediating innate immunity [1,2], are considered to be safe and cost-effective drugs for the health protection of aquacultured fish by providing sufficient protection against a range of fish pathogens [1].

Abbreviations: HK, head kidney; iNOS, inducible nitric oxide synthase; PAMPs, pathogen-associated molecular patterns; PRRs, pattern-recognition receptors; TPS-2, trout stromal cell line from the pronephros; TSS, trout stromal cell line from the spleen.

* Corresponding author. Tel.: +34 987 291 487; fax: +34 987 291917.
E-mail address: alberto.villena@unileon.es (A.J. Villena).
[1] Present address: Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain.

1050-4648/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fsi.2013.07.015

Most immunostimulants are recognized as specific pathogen-associated molecular patterns (PAMPs) [3], which are detected by pattern-recognition receptors (PRRs) in the host cells, as Toll-like [4] and RIG-1 receptors [5]. The binding of these receptors with their respective PAMPs can trigger intracellular signalling pathways leading to the production and release of pro-inflammatory cytokines, which mediate the onset of the innate immune responses [6]. Among the innate effector responses, microbicidal/cytotoxic activities, such as phagocytosis, increased respiratory burst, and release of bactericidal molecules, in macrophages, granulocytes, and other cells of the innate immune system, are of major importance [7].

In a previous paper we have demonstrated that two stromal cell lines derived from the spleen (trout splenic stroma, TSS) and the pronephros (trout pronephric stroma-2, TPS-2) of rainbow trout (*Oncorhynchus mykiss*) were also able to show innate defence responses, including phagocytic activity, and intracellular/extracellular reactive oxygen species and nitric oxide production, and that such responses increased after exposure to the well-known stimulants of the fish immune system lipopolysaccharide (LPS), levamisole, or polyinosinic polycytidylic acid (poly I:C) [8]. Moreover, the two stromal cell lines constitutively expressed the pathogen pattern-recognition receptors (PRRs) Toll-like receptors TLR-3, TLR-5 and TLR-9 genes, and were also able to respond to macrophage-derived factors contained in conditioned supernatants from levamisole- or poly I:C-stimulated macrophage cultures from the head kidney (HK macrophages) of rainbow trout [8].

Those results indicate that the stromal cells of the main lymphohaemopoietic organs of *O. mykiss* may have a role in the development of innate immunity responses being players of defence responses and targets of immunoregulatory factors. Therefore, we suggested that the stromal cell lines may serve to develop *in vitro* methods to select new immunostimulant candidates for aquaculture, which may constitute a cost-effective alternative to *in vivo* experimentation, as they may give more repetitive results under a highly controlled experimental environment [9,10]. In this sense, it has been recently reported the use of a fibroblast cell line (SAF-1) from *Sparus aurata* to demonstrate that *Aloe arborescens* Miller leaf components may be used as an immunostimulant, as such natural plant extracts elicited the expression of different immune-related genes in the SAF-1 cells when co-stimulated with the plant extracts and LPS or poly I:C [11].

To further confirm such idea, we report here the differential expression of various genes related to the regulation of innate immune responses, including pro-inflammatory (IL-1β1, IL-8, TNF-α1, TNF-α2) and immune-suppressing (IL-10) cytokines, of the interferon-induced Mx-1 protein; of enzymes regulating nitric oxide (NO) (iNOS, arginase-2) and eicosanoid (COX-2) production, and of the Toll-like PRRs TLR-3, TLR-5 and TLR-9, in the TPS-2 and TSS cell lines, as well as in cultures of rainbow trout HK macrophages, after their exposure to the same immunostimulants used in the previous paper [8].

2. Materials and methods

2.1. Animals

Healthy specimens of rainbow trout (*O. mykiss*), weighing 150–300 g, were obtained from a fish farm ("Los Leoneses", Castrillo del Porma, León, Spain). They were housed at the Animal Facility of the University of León and maintained in re-circulating freshwater stock tanks (300 L) at 14 °C, with a 12 h light/12 h dark photoperiod and fed daily with a commercial diet (Trouw T6 Classics 3P, Trouw España, Spain).

Prior to any experiment, fish were acclimatized to laboratory conditions for at least 2 weeks, and observed during this period for any clinical sign. All experimental procedures described comply with the Guidelines of the European Union Council (2010/63/EU) for the use of laboratory animals. When necessary, fish were anaesthetized and euthanized with tricaine methanesulphonate (MS-222, Sigma).

2.2. Cell culture

Cell cultures of rainbow trout stromal cell lines from the pronephros (TPS-2) and spleen (TSS) established in our laboratory [12], and of isolated head kidney macrophages (HK macrophages) were used. The main phenotypical and functional features of TPS-2 and TSS cell lines, the methods for their routine maintenance and passages have been described previously [8]. Routine cell culture medium consisted of RPMI-1640 with 25 mM HEPES (Invitrogen) supplemented with 20% FCS, 2 mM L-glutamine (Biochrom), 2.5 mM sodium pyruvate (Merck), 50 μM 2-mercaptoethanol (Merck), 50 μg/ml gentamicin (Biochrom), 2 μg/ml Amphotericin B (Biochrom), and a solution of adenosine, cytosine, guanidine and uridine (Sigma) 0.1% w/v.

HK macrophages were also isolated and cultured as described [8] according to Secombes [13]. Purified plastic-adherent cells from pronephric leukocyte cell suspensions were cultured in 96-well non-pyrogenic polystyrene plates (Corning Inc.) using RPMI-1640 without phenol red with 25 mM HEPES (Invitrogen) and supplemented with 2% FCS, 50 μg/ml gentamicin and 2 μg/ml Amphotericin B.

For the different experiments, the TPS-2 and TSS cell lines and HK macrophages were seeded on 25 cm^2 non-pyrogenic polystyrene flasks (Corning Inc.) and grown to subconfluence at 18 °C under air atmosphere.

2.3. In vitro immunostimulation

The TPS-2 and TSS cell lines and the HK macrophages were stimulated by exposing them to LPS from *Salmonella typhimurium* (Sigma), levamisole (Sigma), or polyinosinic polycytidylic acid (poly I:C, Sigma) using the same concentrations (LPS: was 5 and 50 μg/ml; levamisole: 5 and 25 μg/ml; poly I:C: 1, 50 and 100 μg/ml) previously described to study the effect of these immunostimulants on the innate defence-related responses of the two cell lines and of HK macrophages [8]. The immunostimulants were dissolved to the appropriate final concentration in RPMI-1640 without phenol red containing 25 mM HEPES, 2% FCS, 50 μg/ml gentamicin, and 2 μg/ml Amphotericin B. Subconfluent cell cultures in 25 cm^2 flasks were exposed to the immunostimulants for 4 or 24 h at 18 °C by adding 100 μl/flask of the immunostimulant solution. Control flasks had the same volume of medium without the immunostimulant. All the experiments were run in triplicate, and independently repeated twice.

2.4. Gene expression analysis

The differential expressions of the innate immune-related genes shown in Table 1 were study in the stimulated TPS-2, TSS and HK macrophages cultures using reverse transcriptase-real time quantitative PCR analysis (RT-qPCR).

A preliminary study was carried out to confirm that the different genes included in this study were expressed in the cell lines as well as in the HK macrophages. For this, we used conventional RT-PCR and visualization of the amplification products in ethidium bromide stained agarose gels as previously described [8] (see Supplementary data).

Download English Version:

https://daneshyari.com/en/article/10972073

Download Persian Version:

https://daneshyari.com/article/10972073

Daneshyari.com