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Apoptosis inhibition of Atlantic salmon (*Salmo salar*) peritoneal macrophages by *Piscirickettsia salmonis*

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Abstract

To improve the understanding of the piscirickettsiosis pathogenesis, the in vivo apoptosis modulation of peritoneal macrophages and lymphocytes was studied in juvenile *Salmo salar* intraperitoneally injected with *Piscirickettsia salmonis*. Five fish were sampled at post-exposure days 1, 5, 8 (preclinical), 20 (clinical) and 40 (post-clinical period of the disease), and the leucocytes of their coelomic washings were analysed by flow cytometry (using the JC-1 cationic dye), TUNEL and cytology to detect apoptotic cells. A selective and temporal pattern of apoptosis modulation by *P. salmonis* infection was observed. Apoptosis in lymphocytes was not affected, whereas it was inhibited in macrophages but only during the preclinical stage of the induced piscirickettsiosis. Hence, it is postulated that *P. salmonis* inhibits macrophage apoptosis at the beginning of the disease development to survive, multiply and probably be transported inside these phagocytes; once this process is complete, macrophage apoptosis is no longer inhibited, thus facilitating the exit of the bacteria from the infected cells for continuing their life cycle.

KEYWORDS
apoptosis, fish, leukocytes, *Piscirickettsia*, salmon

1 | INTRODUCTION

Infectious diseases are one of the most important causes of fish mortalities in aquaculture (Austin, 2012; Inglis, Roberts, & Bromage, 1993; Pillay & Kutty, 2005) and are a relevant source of them in wild stocks too (Tompkins, Carver, Jones, Krkošek, & Skerratt, 2015; Winton, 2014). These diseases frequently cause extensive economic losses (Austin, 2012), significantly decrease the availability of high-quality food for human consumption (Lafferty et al., 2015) and generate fish suffering (Bergqvist & Gunnarsson, 2013; Ellis, Berrill, Lines, Turnbull, & Knowles, 2012), among other significant undesirable effects. One of the major fish pathogens is *Piscirickettsia salmonis*, a Gram-negative bacterium which is the aetiological agent of piscirickettsiosis (Fryer, Lannan, Garcés, Larenas, & Smith, 1990; Garcés et al., 1991). This is a septicaemic disease affecting a variety of teleost species (Arkush, Edes, McBride, Adkison, & Hedrick, 2006; Chen et al., 2000; McCarthy et al., 2005; Zrnčić et al., 2015), but is particularly severe in salmonid fish reared in Chilean nearshore sea waters, causing losses for at least US$ 100 million a year in this country (Bravo & Midtlyng, 2007; Smith, Rojas, & Manneschi, 2011). *Piscirickettsia salmonis* is a gamma proteobacterium (Fryer, Lannan, Giovannoni, & Wood, 1992) of a facultative intracellular nature (Mauel, Ware, & Smith, 2008; Mikalsen, Skjærvik, Wiik-Nielsen, Wasmuth, & Colquhoun, 2008) that in its host cells multiplies within cytoplasmic membrane-bound vacuoles (Fryer et al., 1990) and is one of the few, so far described, obligate fish pathogen (Smith, Contreras, et al., 2015). Piscirickettsiosis is a systemic disease (Garcés et al., 1991) and *P. salmonis* infects a variety of cell types in salmonid hosts (Branson & Nieto Díaz-Muñoz, 1991; Larenas, Hidalgo, Garcés, Fryer, & Smith, 1995; Smith, Contreras, et al., 2015), but the main target cells are the macrophages in which this bacterium can survive and replicate extensively (Cubillos et al., 1990; McCarthy et al., 2008).

Apoptosis is a programmed cell death process which is essential in animal development and homoeostasis (Alberts et al., 2015;

Elmore, 2007). This mechanism also plays an important role in the development, maintenance and effect of the immune system including the elimination of infected host cells to avoid multiplication and dissemination of infectious agents (Behar et al., 2011; Han, Zhong, & Zhang, 2011; Medzhitov, 2007). The ultimate goal of all pathogens is to establish a site, or a replicative niche, in the host where the microorganism can multiply (Faherty & Maurelli, 2008), apoptosis manipulation being one of the strategies used by some infectious agents to get this aim. Bacteria (Finlay & McFadden, 2006; Moffatt, Newton, & Newton, 2015), viruses (Finlay & McFadden, 2006; Hay & Kannourakis, 2002) and parasites (Bruchhaus, Roeder, Rennenberg, & Heussler, 2007) can either induce or inhibit apoptosis to provide a survival advantage upon infection of the host (Faherty & Maurelli, 2008). To improve the understanding of piscirickettsiosis pathogenesis, the in vivo apoptosis modulation of peritoneal leucocytes, particularly macrophages and lymphocytes, was studied in experimentally infected Atlantic salmon.

2 | MATERIALS AND METHODS

2.1 | Fish

Juvenile Atlantic salmon (n = 150), mean weight 316 g (SD = 23), which were held in six circular 465-l tanks (25 fish per tank) with a flow-through freshwater system with permanent aeration supply, were used. All the assays described below with these fish were carried out concurrently. This study was approved by the Bioethics Committee of the University of Chile (Permit number 02-2014) which follows the regulations proposed by the National Research Council (USA) described in the Guide for the Care and Use of Laboratory Animals.

2.2 | Bacterium

A *P. salmonis* strain isolated from a diseased Atlantic salmon sampled from a sea site located in the south of Chile having a piscirickettsiosis outbreak was used. After primary isolation and three passages in cell monolayers, the bacterium was preserved in liquid nitrogen until used. It was isolated, grown and titrated in the CHSE-214 cell line (Lannan, Winton, & Fryer, 1984) which was kindly provided by the late Prof. J.L. Fryer (Department of Microbiology, Oregon State University). These cells were cultured at 17°C using Eagle's minimal essential medium (MEM) supplemented with 10% of foetal calf serum (MEM-10) as previously described (Smith, Díaz, et al., 2015).

2.3 | Infectivity trial

This experiment was carried out to know the presentation features of the disease so as to establish its clinical phases as a reference for data interpretation of the concurrently conducted time-course assay explained below in which these salmons had to be killed with the aim of revealing the effect of the bacterium on the apoptotic process at the different clinical stages of the piscirickettsiosis. One hundred fish were allotted into four tanks. Fish of two of those tanks (n = 50) were intraperitoneally (*ip*) inoculated with a bacterial suspension of 1 ml per fish containing $0.3 \times 10^{5.2}$ tissue culture 50% infectious dose ($TCID_{50}$). Fish of the two other tanks were sham-inoculated with a MEM-10 suspension containing uninfected CHSE-214 cells partially disrupted using two freezing–thawing cycles. Fish were observed daily, and clinical signs, mortalities and gross pathology were recorded up to 45 post-exposure (*p.e.*) days. Pure methanol fixed (5 min) kidney smears were obtained from dead fish for Gram staining and for labelling with an immunofluorescence test (FAT) to confirm the presence of *P. salmonis* according to Lannan, Ewing, and Fryer (1991), but using monoclonal instead of polyclonal immunoglobulins and employing DAPI for nuclear identification.

2.4 | In vivo assay for a time-course sampling to evaluate the apoptosis modulation by *P. salmonis* infection

Fifty fish were distributed into two tanks (25 fish per tank) denominated as A and B. Fish of tanks A and B were *ip* injected (1 ml) with *P. salmonis* and sham-inoculated, respectively, as described above in the infectivity trial with whole bacterium. Five fish from each tank were killed using an anaesthetic overdose (Benzocaine at 200 mg/l) at 1, 5, 8, 20 and 40 *p.e.* days to obtain fluids from coelomic washings followed by a necropsy for gross lesions recording.

Given that one fish died during the experiment in tank A, to complete the fish number at the last sampling time, one individual was obtained from the infectivity trial with whole bacterium explained above. Coelomic washings to obtain their peritoneal leucocytes were carried out using a modification of the method described by Afonso, Ellis, and Silva (1997). The procedure was carried out at 17°C and had the following three steps: (1) immediately after euthanasia, as much blood as possible was withdrawn from the caudal vein of the fish tail using a 10-ml syringe fitted, as in all cases, with a 21-G needle; (2) after closing the anal opening with an haemostatic forceps, air followed by MEM-10 was injected (15 ml both) in the coelomic cavity using a 20-ml syringe and (3) following a gentle massage of the fish abdomen for about 30 s, as much as possible of coelomic washing fluid was withdrawn using a 20-ml syringe and collected in lithium heparin spray-coated (150 USP) 10-ml plastic tubes (BD Vacutainer, Franklin Lakes, NJ, USA). Steps 2 and 3 were carried out again, but injecting only 5 ml of air and MEM-10, collecting in total approximately 15 ml of coelomic washing fluid per fish which was then immediately cooled to 10°C. Pictures of selected steps of the coelomic washing procedure are shown in Figure 1.

2.5 | Peritoneal leucocyte apoptosis detection by mitochondrial membrane depolarization, DNA fragmentation and cytological features

All the centrifugations, liquids and incubation steps of this procedure were at 10°C and unless otherwise described centrifugations were

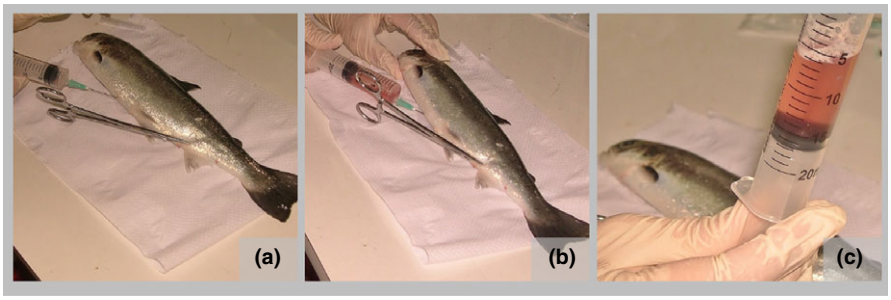

FIGURE 1 Overview of selected steps to obtain coelomic washing fluid in Atlantic salmon (*Salmo salar*). (a) MEM-10 intraperitoneal injection. (b) Coelomic washing fluid withdrawing and (c) collected fluid [Colour figure can be viewed at wileyonlinelibrary.com]

carried out at 400 × ***g*** for 6 min. First and to lyse the red blood cells, the whole volume of each coelomic washing fluid was centrifuged, and the pellet resuspended in distilled water (4.5 ml). After a short incubation period (8 s), 0.5 ml of 10× PBS pH 7.2 was added (adjusted to have 355 mOsm/l in the 1× solution). The resulting suspension was centrifuged and the pellet resuspended in 5 ml of MEM-10. This volume was divided into four fractions denoted F1, F2, F3 (1 ml each) and F4 (2 ml). The first three fractions were used to be analysed by flow cytometry to detect mitochondrial membrane depolarization, while F4 to prepare paraffin blocks for immunochemistry, to detect DNA fragmentation and for cytology. F1 was used unstained as a background control. F2 was treated first with 35 mM carbonyl cyanide 3-chlorophenylhydrazone (CCCP; Sigma-Aldrich, St. Louis, MO, USA) for 20 min (to induce a dissipation of the mitochondrial membrane potential as a positive control), and then it was incubated at dark for 20 min with 0.25 μg/ml of the cyanine dye JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetra-ethylbenzimidazolo-carbo-cyanine iodide purchased from Thermo Fisher Scientific, Waltham, MA, USA). F3 was JC-1 incubated as already explained. After JC-1 incubation, F2 and F3 were centrifuged and the pellet resuspended in MEM-10 (200 μl). Treated samples (F1, F2 and F3) were then immediately analysed by flow cytometry (BD FACSCanto II, BD Biosciences, Franklin Lakes, NJ, USA); macrophage and lymphocyte populations were recognized and then gated (i.e., selected) by the size (forward scatter or FSC) and complexity (side scatter or SSC) of these cells in the respective plots as previously described (Kfoury, Kuroda, Nakayasu, Fukuda, & Okamoto, 1999; Macey, 2007). In turn, macrophages and/or lymphocytes that emitted green fluorescence, therefore showing mitochondrial membrane depolarization (Binet, Doyle, Williamson, & Schlegel, 2014), were considered as apoptotic cells. Regarding the treatment of the F4 fraction, after a centrifugation step (500 × ***g*** for 10 min) its pellet was fixed with 0.8 ml of Shandon Glyo-fixx (Thermo Scientific, Waltham, MA, USA) for 4 h at room temperature (*r.t.*) and then centrifuged again (1,000 × ***g*** for 10 min) to wash it with a solution containing ethanol and pH 7.2 PBS (1:1v/v). This suspension was centrifuged (1,000 × ***g*** for 10 min), and its pellet was resuspended in 100 μl of 2% PBS (pH 7.2) diluted agarose (Ultrapure agarose; Invitrogen, Carlsbad, CA, USA) melted at 60°C. The agarose-pellet was cooled first at *r.t.* for 10 min and then at −20°C for 5 min to be paraffin embedded using standard histological methodology. Three sections (3 μm thick) were obtained per each paraffin block. First section was FAT labelled as previously explained to confirm by a qualitative observation the presence of *P. salmonis* in the peritoneal leucocytes of the infected fish. The second of them was used for immunochemistry following the manufacturers' instructions of a kit for *in situ* apoptosis detection via DNA fragmentation by a terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine (dUTP) nick end labelling (TUNEL) assay (Apoptag S7100; Millipore Corporation, Temecula, CA, USA). Atlantic salmon gut sections were also included as a positive apoptosis control of this test. The third histological section was H and E stained and used for cytological analysis. Besides the cytological observations, both the TUNEL and the H and E stained preparations were used to record the percentage of macrophages suffering apoptosis in each microscopic section. For this last goal, 100 of these cells were scrutinized in each slide (TUNEL and H and E staining); sections with fewer than 100 macrophages were not considered. To these examinations, the whole area of these sections was observed under a light microscope at 400× magnification and in only some selected fields, when required, at 1,000× amplification. With TUNEL, macrophages were counted as apoptotic when they were immunoperoxidase-stained (brown), and with H and E staining when they exhibited the typical cytological features of apoptosis characterized by cell shrinkage, pyknosis (Kerr, Wyllie, & Currie, 1972) as well as eosinophilic cytoplasm and chromatin fragmentation (Elmore, 2007; Kuhlmann, Bitsch, Stadelmann, Siebert, & Brück, 2001). Selected apoptotic macrophages detected by TUNEL and cytology are shown in Figure 2. Statistical comparison of the median of the apoptotic leucocyte percentage in each case was carried out using the nonparametric Kruskal–Wallis test.

3 | RESULTS

In the infectivity trial, the piscirickettsiosis was reproduced in several of the experimentally infected fish. The first of these fish died at 18 *p.e.* day, total cumulative mortality reaching 24% at 28 *p.e.* day (Figure 3). Earliest disease manifestation took place at day 14 *p.e.*, and it was characterized by the presence of faecal casts in the bottom of the fish tanks, which was indicative that at least some of these animals had enteritis. From day 17 *p.e.*, fish exhibited anorexia, lethargy, dorsal skin darkening and reddening of the pectoral fin bases. Dead fish at necropsy showed pale gills and petechiae and (or) ecchymoses in the skin of the lateral sides of the trunk, including the base of the pectoral and pelvic fins. Internally, these fish exhibited ascites, liver paleness, kidney, intestine and swim bladder congestion, muscles of

the abdominal wall with petechiae, as well as heart with atrial distension and petechiae in the pericardium. In kidney smears from all the dead fish, the presence of *P. salmonis* was confirmed by FAT and only organisms compatible with this bacterium were seen with Gram staining. There were no mortalities or clinical signs in the non-inoculated control fish. According to the above results, under these experimental conditions the preclinical, clinical and post-clinical periods of the disease occurred at *p.e.* days 1–13, 14–28 and 29–45, respectively, in the affected animals (Figure 3).

With respect to the assay for the time-course sampling, no lesions were found at necropsies of fish injected with *P. salmonis* in sampling times carried out at 1, 5, 8 (preclinical phase) and 40 (post-clinical phase) *p.e.* days. Nevertheless, at day 20 *p.e.* four of the five sampled fish exhibited some or all of the following gross lesions: petechiae in skin of the trunk sides, paleness of liver, congestion of kidney, gut and pyloric caeca, petechiae and/or congestion of skeletal muscle of the abdominal wall, dilatation of atrium as well as petechiae and ecchymoses in the pericardium.

In relation to the evaluation of the apoptosis modulation by *P. salmonis* through flow cytometry of leucocyte populations from peritoneal washings, it was found that the apoptosis percentage of the lymphocyte populations was not significantly affected ($p > .05$) at any sampling time. Nevertheless, results at this respect with macrophage populations were different. Although the fraction (%) of apoptotic macrophages did not vary at the clinical and post-clinical phases compared to the control, it was significantly lower ($p \leq .05$) in the treated group in two (days 5 and 8 *p.e.*) of the three sampling points of the preclinical phase of the disease (Figure 4).

With respect to the FAT of the peritoneal leucocyte sections, *P. salmonis* was found in the infected fish at the preclinical and clinical stage in the macrophage cytoplasm and high bacterial numbers were particularly seen at 5 and 8 *p.e.* days as illustrated in Figure 2.

Regarding the immunochemistry with TUNEL, in the first place it should be noticed that in only four of the five slides of each sampling time 100 macrophages could be counted (apoptotic or not) and then considered valid (same consideration occurred with cytology). As it was the case with flow cytometry, TUNEL results showed a lower apoptotic percentage ($p \leq .05$) at days 5 and 8 *p.e.* with *P. salmonis* (Figure 4). The apoptosis response at day 40 *p.e.* was assessed only by flow cytometry and cytology but not by TUNEL.

With reference to the cytological results, excepting the lack of statistical significance at day 5 *p.e.* with *P. salmonis*, they exhibited the same pattern as shown by the other two methods used to detect apoptosis (Figure 4).

4 | DISCUSSION

The apoptotic cell frequency in some macrophage and lymphocyte populations was assessed in Atlantic salmon after being experimentally exposed to *P. salmonis*. Cumulative mortality in fish inoculated with the bacterium reached 24% and the clinical signs and the pathological manifestations of the diseased fish were consistent with those communicated by several authors for piscirickettsiosis (Arkush & Bartholomew, 2011; Branson & Nieto Díaz-Muñoz, 1991; Cvitanich, Garate, & Smith, 1991; Fryer & Hedrick, 2003; Monasterio, 2008). Based on epidemiological data, it has been reported that the cumulative mortality of the field outbreaks of piscirickettsiosis in Chile ranges from 20% to 30% (Sernapesca 2012). The close

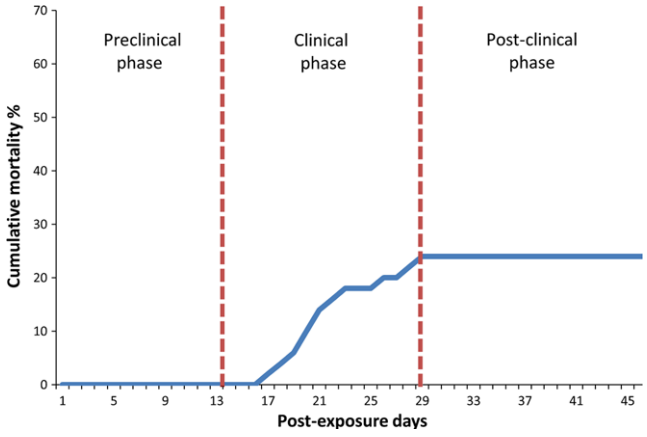

FIGURE 3 Cumulative mortality (%) of Atlantic salmon (*Salmo salar*) intraperitoneally inoculated with *Piscirickettsia salmonis* ($0.3 \times 10^{5.2}$ $TCID_{50}$ per fish) [Colour figure can be viewed at wileyonlinelibrary.com]

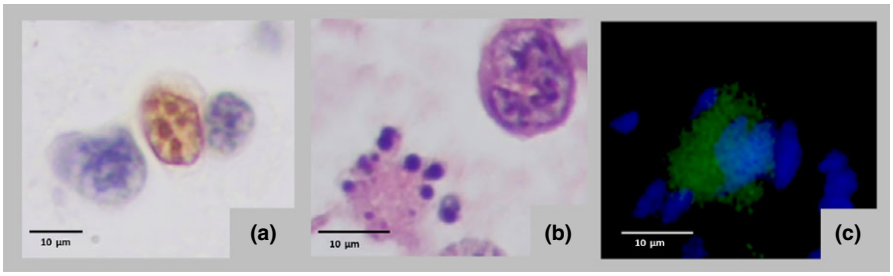

FIGURE 2 Histological sections of Atlantic salmon (*Salmo salar*) paraffin-embedded coelomic washing pellets. (a) Macrophages, one of them immunoperoxidase-stained (brown) and therefore identified as apoptotic (TUNEL, 1,000×). (b) Macrophages, one of them apoptotic showing shrinkage, karyorrhexis and apoptotic bodies (H and E, 1,000×). (c) Macrophage showing a high quantity of *Piscirickettsia salmonis* (green fluorescence) in its cytoplasm (FAT, 400×) [Colour figure can be viewed at wileyonlinelibrary.com]

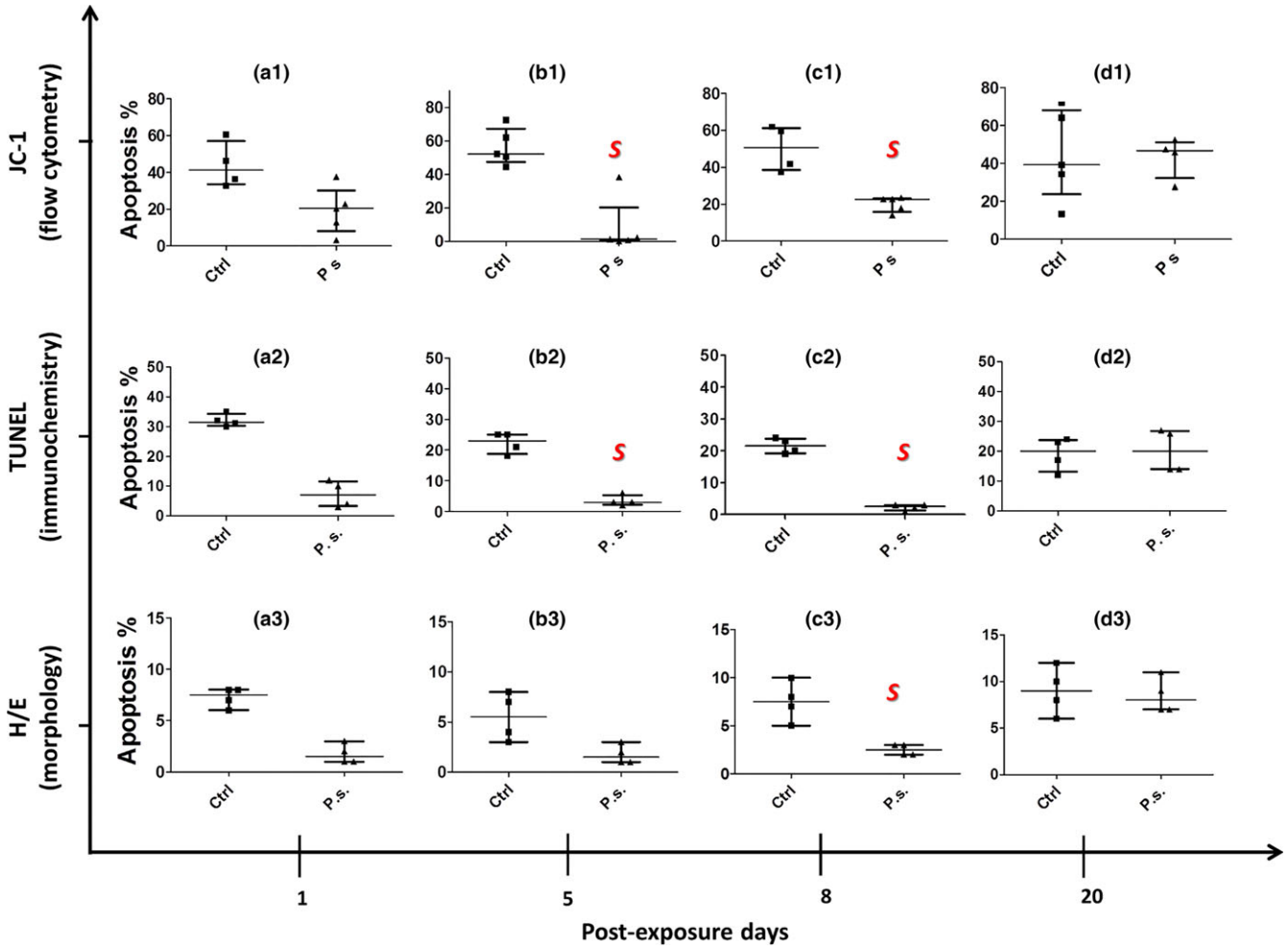

FIGURE 4 Median and first and second quartile of apoptosis (%) of macrophage populations from coelomic washings of Atlantic salmon (*Salmo salar*) injected with *Piscirickettsia salmonis* (Ps) at post-exposure (*p.e.*) days 1, 5, 8 and 20. Macrophage apoptosis (% median) was significantly lower ($p \leq .05$) compared to its control (Ctrl) —which is indicated with the letter *S*—at *p.e.* days 5 (Graphs b1 and b2) and 8 (Graphs c1, c2 and c3) in the fish infected with *P. salmonis*. Results at *p.e.* day 40 (not shown), followed the same pattern as at *p.e.* day 20 [Colour figure can be viewed at wileyonlinelibrary.com]

resemblance of the disease presentation between the inoculated and the field exposed fish suggests that this experimental model was useful to understand the way in which the apoptosis modulation of *P. salmonis* occurs when the disease takes place in natural conditions.

As to the apoptosis modulation of the leucocyte populations of fish infected with *P. salmonis*, no such phenomenon occurred in macrophages at the clinical and post-clinical phases of the experimentally induced piscirickettsiosis and throughout all the disease development in the case of lymphocytes. Nevertheless, the pathogen was able to decrease the apoptosis frequency of macrophages during the preclinical stage of this disease. This conclusion is mainly supported by the significant lower apoptosis percentage ($p \leq .05$) of macrophages of the infected fish detected by flow cytometry and TUNEL in two of the three sampling points of the preclinical phase of the disease (i.e., at 5 and 8 *p.e.* days) and in one of these points (8 *p.e.* day) in the cytological analysis (Figure 4). In addition, in the preclinical sampling points that no statistical differences were found, the median of the infected animals was clearly and consistently lower than their controls (Figure 4). Apoptosis detection was lower by cytology than by flow cytometry and TUNEL, but, as mentioned, it followed the same pattern shown by these two methods compared with their respective controls (Figure 4). It is widely known that the search for apoptotic cells by light microscopy based only on morphological features is a method with low sensitivity, but it is a reliable way to confirm whether the results obtained by other procedures are consistent with the expected cell phenotypical expression observed directly by optical means (Archana, Yogesh, & Kumaraswamy, 2013). Results showed that *P. salmonis* modulate the apoptosis rate of some specific leucocyte populations since the apoptosis inhibition occurred in macrophages and not in lymphocytes.

It has been extensively described in a number of infectious diseases caused either by extra or intracellular bacteria that the modification of the death modality of a variety of target eukaryotic cells is an important feature of their pathogenesis (Aguilo et al., 2013; Fahrerty & Maurelli, 2008; Favaloro, Allocati, Graziano, Di Ilio, & De

Laurenzi, 2012; Häcker, Kirschnek, & Fischer, 2006; He & Amer, 2014; McCracken, Kinkead, McCaffrey, & Allen, 2016; Pacios et al., 2013; Silva, 2010). More specifically, subversion of the macrophage mode of death is a common feature of several intracellular pathogens of humans and other vertebrates including fish (Behar, Divangahi, & Remold, 2010; Sepulcre, Muñoz, Roca, López-Muñoz, & Mulero, 2010). Furthermore, as found in the present work, the inhibitory effect on monocyte/macrophage apoptosis, at least in some stage of the infective cycle, has been previously reported in other intracellular bacteria such as *Legionella pneumophila* (Banga et al., 2007), *Coxiella burnetii* (Klingenbeck, Eckart, Berens, & Lührmann, 2013), *Brucella* spp. (Gross, Terraza, Ouahrani-Bettache, Liautard, & Dornand, 2000) *Neisseria* spp. (Tunbridge et al., 2006) and *Mycobacterium tuberculosis* (Behar et al., 2010; Briken & Miller, 2008).

The molecular bases that explain the interfering mechanisms of the apoptosis by *P. salmonis* are unknown, but it has been described that several pro-apoptotic genes are downregulated in Atlantic salmon tissues after being experimentally infected with this bacterium (Rise et al., 2004; Tacchi et al., 2011), which is in agreement in terms of the apoptosis inhibition observed here. Fish tissues were examined at *p.e.* days 2 and 14 in the works of Rise et al. (2004) and Tacchi et al. (2011), respectively, and some of the pro-apoptotic downregulated genes were as follows: death-associated protein 1b (Rise et al., 2004), Bcl 10-interacting CARD protein, gsk-3 binding protein and Thap domain-containing apoptosis-associated protein 1 (Tacchi et al., 2011). It is also possible that the exotoxins found in the extracellular products (Rojas et al., 2013) and also in the outer membrane vesicles of *P. salmonis* (Oliver et al., 2015) may play a role in modulating the apoptosis of its host target cells.

To our knowledge, the only previous work devoted to study the apoptosis modulation by *P. salmonis* was carried out by Rojas et al. (2010) who infected cell cultures of a monocyte/macrophage-like cell line (RTS11) which, in turn, is derived from rainbow trout (*Oncorhynchus mykiss*) spleen (Ganassin & Bols, 1998). In contrast to the present work, these authors found a consistent significant apoptosis induction using several detection methods in the three sampling points tested, that is, at 2, 5 and 10 *p.e.* days. This variation in the results may probably be explained by the different models used in those works, that is in vitro vs. in vivo assays.

As it occurs with the outcome of the interplay between other pathogenic bacteria and their respective hosts, particularly those with an intracellular style of life, it was found in this work that the in vivo infection of *P. salmonis* in Atlantic salmon causes a modification in the apoptosis rate of some specific cell populations and that this phenomenon has a temporal pattern. Apoptosis frequency was diminished in macrophages while it remained unchanged in lymphocytes. In turn, this inhibition had a temporal pattern occurring only during the preclinical stage and not in the clinical and post-clinical phases of the disease. In addition, it was observed that *P. salmonis* replicated in a large quantity within macrophages during the preclinical stage of the disease. According to these results, we postulate that *P. salmonis* inhibits macrophage apoptosis at the preclinical stage of piscirickettsiosis to survive, multiply and probably be transported inside these phagocytes at the beginning of the disease development; once this process has finished the inhibition of the apoptosis in macrophages no longer occurs, which would facilitate the exit of the bacteria from the infected cells for continuing their life cycle.

Piscirickettsiosis results in a significant socioeconomic and fish welfare problem, particularly in salmonid aquaculture in Chile, and new information that increases our understanding of its pathogenesis, such as that presented in this study, may assist in the development of efficient strategies or products, such as vaccines and/or therapeutic drugs, to prevent or control this deleterious fish disease.

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