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Characterization of myocardial lesions associated with cardiomyopathy syndrome in Atlantic salmon, *Salmo salar* L., using laser capture microdissection

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Abstract

Cardiomyopathy syndrome (CMS) in Atlantic salmon, *Salmo salar* L., is characterized by focal infiltration in the spongy myocardium and endocardium of the heart. The origin of the mononuclear infiltrate is unknown. Using experimentally infected fish, we investigated localization of the causative agent, piscine myocarditis virus (PMCV), within the heart and characterized the cell population associated with myocardial lesions. Cellular and transcriptional characteristics in the lesions were compared with adjacent non-infiltrated tissues using laser capture microdissection, RT-qPCR and immunohistochemistry. Our results reveal that PMCV is almost exclusively present in myocardial lesions. The inflammatory infiltrate comprises a variety of leucocyte populations, including T cells, B cells, MHC class II$^+$ and CD83$^+$ cells, most likely of the macrophage line. Correlation analyses demonstrated co-ordinated leucocyte activity at the site of the virus infection. Cellular proliferation and/or DNA repair was demonstrated within the myocardial lesions. Different cell populations, mainly myocytes, stained positive for proliferating cell nuclear antigen (PCNA). Densities of endothelial cells and fibroblasts were not significantly increased. The simultaneous presence of PMCV and various inflammatory cells in all myocardial lesions analysed may indicate that both viral lytic and immunopathological effects may contribute to the pathogenesis of CMS.

Keywords: immunoglobulin T, inflammatory cells, leucocytes, myocarditis, piscine myocarditis virus, piscine reovirus.

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Introduction

Cardiomyopathy syndrome (CMS) is a viral disease of farmed Atlantic salmon, *Salmo salar* L., first described in Norway in 1985 (Amin & Trasti 1988), followed by Scotland (Rodger & Turnbull 2000) and the Faroe Islands (Poppe & Seierstad 2003). Cases resembling CMS have also been reported from Canada (Brocklebank & Raverty 2002). A double-stranded RNA virus probably belonging to the Totiviridae family and named piscine myocarditis virus (PMCV) was recently identified as the causative agent of CMS (Haugland *et al.* 2011; Lovoll *et al.* 2010). The disease has significant economic impact on aquaculture as it primarily affects adult Atlantic salmon close to harvest, with total annual direct losses to the Norwegian salmon industry estimated as €4.5 to 8.8 million in 2003 (Brun *et al.* 2003). With

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the increase in national production of salmon in recent years, present losses are probably even higher. Of approximately 500 active fish farms nationally (www.fdir.no), between 53 and 85 have been annually diagnosed with CMS during the last decade.

Cardiomyopathy syndrome is a chronic disease that develops over several months prior to the clinical phase (Ferguson, Poppe & Speare 1990). Histopathologically, CMS is characterized by massive cell infiltration with subsequent cardiomyocyte degeneration and necrosis in the spongy myocardium of the ventricle and atrium. The compact myocardium is usually unaffected. The infiltrates comprise mainly mononuclear cells, morphologically referred to as lymphocytes and macrophages (Bruno & Ellis 1988; Fritsvold *et al.* 2009). Another severe disease of farmed Atlantic salmon in which cardiac lesions are prominent is heart and skeletal muscle inflammation (HSMI). HSMI is considered an important differential diagnosis for CMS, but is characterized histopathologically by inflammatory lesions in both spongy and compact myocardium of the ventricle. In addition, HSMI is usually characterized by myositis and necrosis of red skeletal muscle (Kongtorp, Taksdal & Lyngoy 2004). HSMI is associated with piscine reovirus (PRV), a double-stranded RNA virus (Palacios *et al.* 2010).

In mammals, myocarditis and inflammatory cardiomyopathy may arise from various causes, of which viral infections are the most frequent in developed countries (Thiene *et al.* 2005; Blauwet & Cooper 2010). The pathogenesis of virus-induced myocarditis can generally be differentiated into three phases. The early phase relates to viral entry into cardiomyocytes, followed by innate and acquired immune reactions. If the host's immune system is able to effectively limit the infection, the inflammation often resolves. However, progression into chronic forms associated with compromised cardiac function may occur (Kuhl & Schultheiss 2009; Blauwet & Cooper 2010). Autoimmunity or direct cytotoxicity because of persistent virus infection has been proposed to account for the myocyte damage and progression into chronic myocarditis (Calabrese & Thiene 2003). Manifestation of myocarditis varies with both virus and host species, but generally seems to depend on different contributions from viral lytic effects, immune-mediated pathology and developed autoimmunity (Maisch *et al.* 2002; Sagar, Liu & Cooper 2012). In murine models, mononuclear cells, primarily monocytes, macrophages and T lymphocytes dominate (>70%) the inflammatory infiltrate in myocarditis lesions (Pummerer *et al.* 1991).

In this study, we investigated the myocardial lesions associated with CMS at a late stage in the infection. We assessed the presence of the viral pathogens PMCV and PRV and characterized the inflammatory cells within the myocardial lesions using immunohistochemistry and a combination of laser capture microdissection (LCM) and reverse-transcriptase quantitative PCR (RT-qPCR).

Materials and methods

Tissue samples

Cardiac tissues from challenged and prechallenged fish were obtained from a previously reported challenge study (Fritsvold *et al.* 2009). Briefly, 200 unvaccinated post-smolt Atlantic salmon were intraperitoneally injected with tissue homogenate (hearts and kidney) supernatant from fish diagnosed with CMS. The challenge material was later shown to contain both PMCV and PRV (Lovoll *et al.* 2010). However, the challenged fish only developed lesions consistent with CMS. These lesions were identified in the atrium from 6 weeks post-challenge (wpc), with subsequent progression to the spongy myocardium of the ventricle 9 wpc. The most severe inflammatory changes were observed in four individual fish at 30, 33 and 42 wpc (Fig. 1a,b). Cardiac tissues were sampled prior to challenge and every third-week post-challenge for a period of 42 weeks. The hearts were divided in two by a longitudinal incision, and the respective halves either fixed in 10% neutral phosphate-buffered formalin or frozen at −80 °C. Formalin-fixed tissues were processed for histological examination following standard procedures (Bancroft & Stevens 1990) and stained with haematoxylin and eosin (H&E). Our study focused on the ventricles from three hearts displaying severe inflammation, one sampled 30 wpc and two sampled 33 wpc. These hearts were further investigated by immunohistochemistry, special histological staining, and by combined LCM and RT-qPCR.

Immunohistochemistry

Immunohistochemistry was performed using various antisera for the detection of CD3ε (diluted

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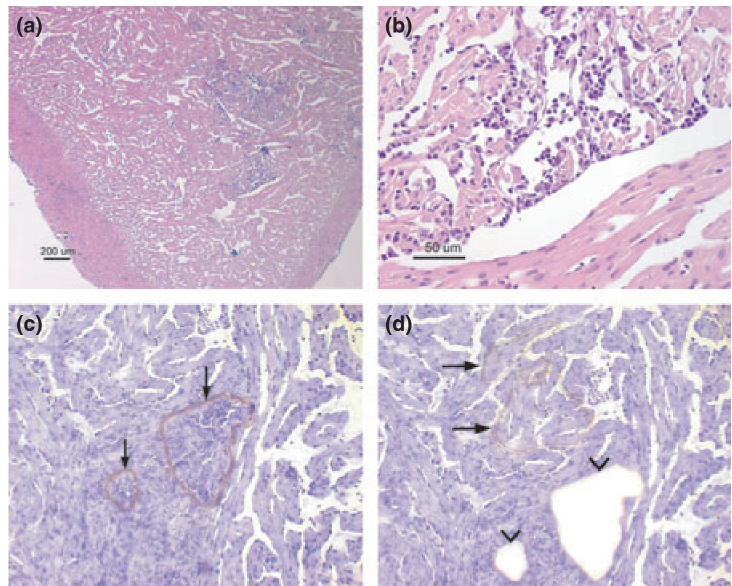

Figure 1 (a) Micrograph of a heart section with typical, focal cardiomyopathy syndrome (CMS) lesions in the spongy layers of the ventricle (H&E). (b) Micrograph of a myocardial lesion with mononuclear cell infiltration (H&E). (c) Micrograph of the spongy layers of a ventricle with typical myocardial lesions obtained for laser capture microdissection (Hx) showing the two lesions to be captured (brown line, vertical arrows). (d) Following removal of the lesion (arrowheads), an adjacent apparently normal area is outlined to serve as normal control tissue (light brown line, horizontal arrows; Hx).

1:300; Koppang *et al.* 2010), IgM (1:4000; kindly provided by Dr O. Sunyer, University of Pennsylvania, Philadelphia, PA, USA), MHC class II molecules (1:1000; Koppang *et al.* 2003), proliferating cell nuclear antigen (PCNA; 1:150; α-PCNA, No. M0879; Dako) and endothelial cells (1:100; Aamelfot *et al.* 2012). Target proteins were visualized using aminoethylcarbazole (AEC) or 3,3′-diaminobenzidine (DAB) substrate. Staining procedures are provided in detail in the respective citations. Cardiac tissues from fish sampled prior to CMS challenge (Fritsvold *et al.* 2009) were used as negative controls.

Special histological staining

The selected hearts were sectioned and stained for collagen with the van Gieson stain (Bancroft & Stevens 1990). Cardiac tissues from fish sampled prior to CMS challenge (Fritsvold *et al.* 2009) were used as negative controls.

Laser capture microdissection

Longitudinal cryosections (7 μm in thickness) of the hearts were cut, mounted on membrane slides (No. 50103; Molecular Machines and Industries) and stained with RNase-free haematoxylin. Ventricular tissues displaying distinct myocardial lesions characteristic for CMS were laser capture microdissected (Fig. 1c) with the SLμCut microdissection system as described previously (Haugarvoll *et al.* 2008). For each infiltrated area (lesion), an adjacent apparently normal area of approximately the same size was dissected for pairwise comparison (Fig. 1d). A total of 22 paired areas, ranging from 0.011 to 0.044 mm^2, were cut and collected into separate tubes (Table S1). Ten samples were dissected from hearts 1 and 2 (33 wpc). Two samples were dissected from heart 3 (30 wpc). The mean difference between the areas sampled from lesions, and the corresponding normal areas was 0.9%.

RNA extraction, reverse transcription (RT) and qPCR

The microdissected samples were examined with RT-qPCR assays for PMCV, PRV, T-cell receptor (TCR), MHC class II, membrane-bound immunoglobulin M (sIgM), membrane-bound immunoglobulin T isotype B ($sIgT_B$) and CD83. Elongation factor 1-alpha (EF1α) was selected as reference gene for the transcription data analysis (Olsvik *et al.* 2005). Primer and probe sequences are listed in Table 1. Total RNA was isolated using the PicoPureTM RNA Isolation Kit (Arcturus Bioscience), which is optimized for use with cells acquired by LCM on CapSure® LCM Caps. The isolation was performed according to the manufacturer's protocol, including DNase treatment. The eluted RNA (12 μL) was reverse transcribed using Sensiscript® RT kit (Qiagen) in a volume of 20 μL according to the

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Table 1 Primers and probes used for the detection and amplification of viral RNA and genes (all 5′-3′)

Gene	Forward primer (900 nM)	Reverse primer (900 nM)	MGB probe (200 nM)	Reference
PMCV	TTCCAAACAATTCGAGAAGCG	ACCTGCCATTTTCCCCTCTT	CCGGGTAAAGTATTTGCGTC	Lovoll *et al.* (2010)
PRV	TGCTAACACTCCAGGAGTCATTG	TGAATCCGCTGCAGATGAGTA	CGCCGGTAGCTCT	Palacios *et al.* (2010)
MHCII	CTCACTGAGCCCATGGTGTAT	GAGTCCTGCCAAGGCTAAGATG	CTGGGACCCGTCCCTG	Haugarvoll *et al.* (2008)
TCRα	GACAGCTACTACAGCCAGGTT	CAGAATGGTCAGGGATAGGAAGTT	ACACAGATGCAAAGATC	Moore *et al.* (2005)
sIgM	CTACAAGAGGGAGACCGGAG	AGGGTCACCGTATTATCACTAGTTT	TCCACAGCGTCCATCTGTCTTTC	Present study
sIgT$_B$	GAATGTTTGGGACACGGAAG	TCACATATCTTGACATGAGTTACCC	CGCCGTCAGGCACGACAGCTT	Present study
CD83	GTGGCGGCATTGCTGATATT	CTTGTGGATACTTCTTACTCCTTTGCA	CACCATCAGCTATGTCATCC	Haugarvoll *et al.* (2006)
EF1αA	CCCCTCCAGGACGTTTACAAA	CACACGGCCCACAGGTACA	ATCGGTGGTATTGGAAC	Olsvik *et al.* (2005)

manufacturer's instructions. Aliquots of 1 μL cDNA from each sample were used for qPCR with either TaqMan® Gene Expression Master Mix (PMCV, PRV, sIgM and sIgT$_B$; Applied Biosystems) in a final volume of 10 μL *or* Platinum qPCR SuperMix-UDG (TCR, MHC class II, CD83 and EF1α; Invitrogen) in a final volume of 20 μL. The RT-qPCR was performed using Stratagene MX3005P at the following settings: 2 min at 50 °C, 10 min at 95 °C, 49 cycles of 15 s at 95 °C and 60 s at 60 °C.

Statistical analysis

Ct values from myocardial lesions and corresponding normal areas were compared using the paired Mann–Whitney *U*-test. Correlation between Ct values was examined within and without the normal tissue areas using the Kendall tau nonparametric correlation test. Statistical analyses were performed using R 2.11.1 (R Development Core Team 2010) and JMP (SAS Institute Inc.). All RT-qPCR assays were run in duplicate with mean Ct values used for statistical analysis. When only one parallel gave a Ct value, this value was used in the analysis. When no Ct value was produced, Ct values were arbitrarily set to 45 to include the sample in the statistical analysis. Both the Mann–Whitney and the Kendall correlation tests are rank tests, and hence, the introduction of the value 45 gives a number that is higher than all observed values. Normalization is critical when attempting to quantitatively compare gene transcription levels between biological samples. As LCM yields low quantities of RNA, quantitation of RNA alone might produce a large variance that prohibits normalization against RNA (Erickson *et al.* 2009). However, owing to the introduction of the above mentioned arbitrary values, normalization of target gene Ct values against reference gene Ct values was not performed. The validity of omitting normalization against a reference gene was tested by *t*-test and Pearson correlation test on the portion of paired samples with target Ct values <45. These data were normalized against EF1α using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001). As an alternative to normalization, lesions and adjacent normal tissue areas were compared directly based on the equality in size of the dissected tissue areas. Alternatively, Ct values were normalized against tissue area using the

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following formula: Ct-norm = $-\log_2(2^{-Ct}/\text{area})$ prior to Kendall tau analysis.

Results

Immunohistochemistry demonstrated several leucocyte populations in the lesions

The salmon investigated in this study developed severe myocardial lesions in the spongy layer of the ventricle as previously described by Fritsvold *et al.* (2009). T cells, stained by CD3-antiserum (Fig. 2a) and IgM^+ cells (Fig. 2b), were abundant in the lesions. IgM staining also resulted in a more disseminated staining pattern, compatible with the detection of extracellular (humoral) IgM. MHC class II^+ cells were not as abundant as T cells or IgM^+ cells (Fig. 2c). $PCNA^+$ cells were observed within or close to myocardial lesions (Fig. 2d), and the dominating cell type displayed large oval nuclei indicative of cardiac myocytes (Fig. 2e). Endothelial labelling revealed the lesions to be surrounded by a more or less intact endothelial lining with a non-elevated number of endothelial cells (Fig. 2f). Van Gieson staining demonstrated the absence or low presence of collagen, suggesting little or no increase in collagen-producing cells such as fibroblasts as a result of the inflammation.

In normal tissues adjacent to myocardial lesions, a limited number of IgM^+ and $PCNA^+$ cells were seen, although in much lower numbers than within the lesions. T cells and MHC class II^+ cells were rarely seen. T cells and IgM^+ cells could occasionally be seen in the lumen of blood vessels. MHC class II^+ and $PCNA^+$ cells were not observed intravascularly.

PMCV but not PRV was found in laser-captured lesions from spongy myocardium

The paired samples of myocardial lesions and normal tissue were compared for levels of PMCV, PRV and immune gene mRNAs, respectively (Fig. 3). PMCV was detected in all 22 samples

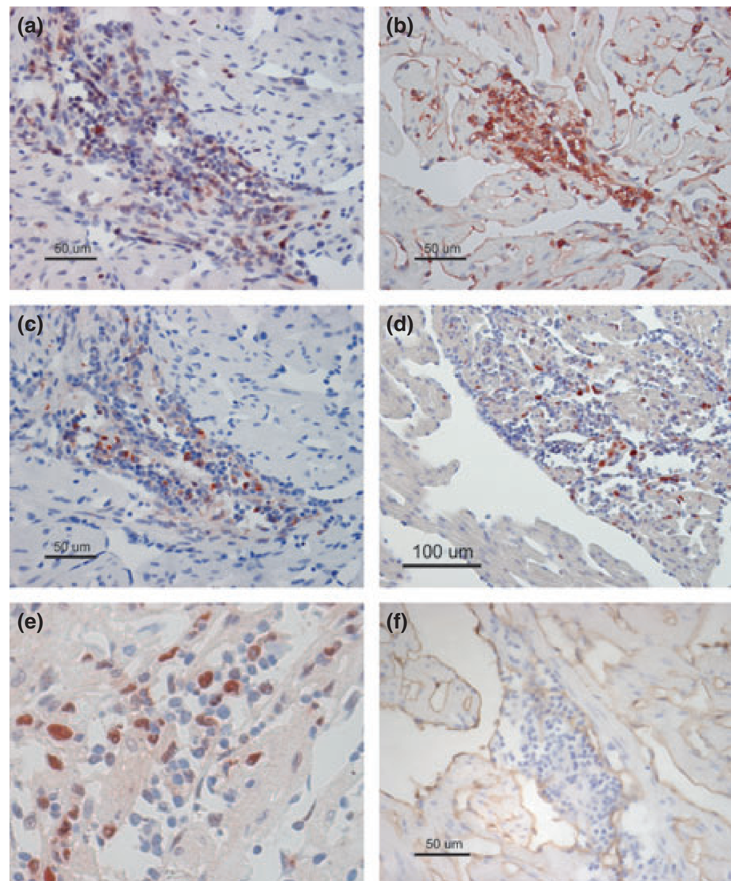

Figure 2 Micrographs of the spongy layer of heart ventricles 33 weeks after experimental infection with piscine myocarditis virus (PMCV). Immunhistochemical staining for (a) CD3, (b) IgM, (c) MHC class II, (d) PCNA, (e) close-up proliferating cell nuclear antigen (PCNA) and (f) endothelial cells. Target proteins were visualised using aminoethylcarbazole (AEC) or 3,3′-diaminobenzidine (DAB) substrate (red and brown, respectively) counterstained with Meyer's hematoxylin (Hx) solution (blue).

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lesions, but in only one sample from normal tissues. In the single positive sample from normal tissue, PMCV was found in a lower amount compared to the corresponding adjacent lesion (Ct 35 vs. Ct 30). PRV was not detected in any of the captured samples from lesions or normal tissues.

Immune gene transcripts in laser-captured lesions were significantly elevated

Mann–Whitney *U*-test showed that transcript levels of TCR ($P < 0.001$), MHC class II ($P < 0.001$), sIgM ($P < 0.001$) and CD83 ($P < 0.001$) were consistently higher (*i.e.* lower Ct) in lesions compared with corresponding normal tissues (Fig. 4a).

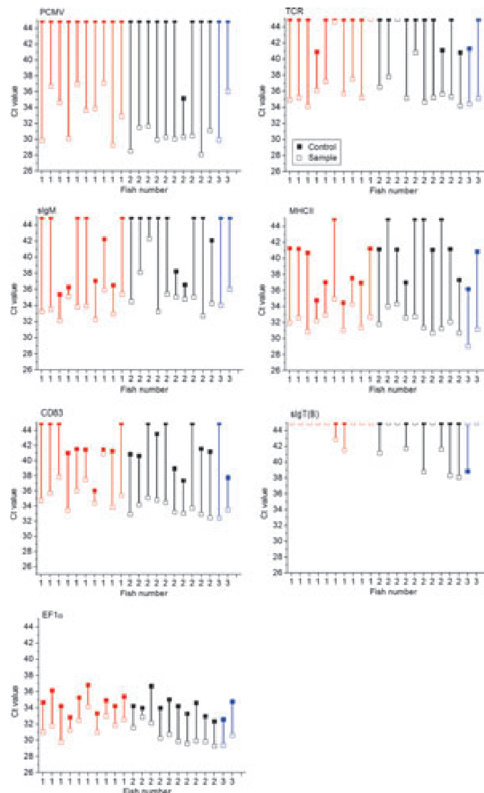

Figure 3 Transcript levels of piscine myocarditis virus (PMCV), T-cell receptor (TCR), sIgM, MHC class II, CD83, $sIgT_B$ and EF1α in laser capture samples microdissected from cardiomyopathy syndrome (CMS) lesions (open square) compared with corresponding normal tissues (filled square). Negative samples are shown as Ct = 45 (half square). Twenty two paired samples were dissected, ten areas from fish number 1 (red) and 2 (black) and two areas from fish number 3 (blue). All RT-qPCR assays were run in duplicate, and the mean value is presented.

Transcripts of $sIgT_B$ were detected in eight lesions but in only one normal tissue sample, all in relatively low amounts.

Area and reference gene normalized gene expression data did not deviate

EF1α was selected for the normalization of gene transcription. EF1α mRNA transcript levels were, however, consistently and significantly ($P < 0.001$, Mann–Whitney *U*-test) higher in samples from lesions compared to normal tissues. This is most probably due to higher cell densities in the inflammatory lesions. The magnitude of the differences was, however, significantly higher for target genes compared to the reference gene. Testing of the validity of omission of normalization against the reference gene demonstrated that the data did not deviate from the area-normalized results. We therefore decided to present the area-normalized gene transcription data.

Correlation between PMCV and immune genes

Kendall tau testing was performed to assess the correlation of PMCV levels and transcription of various immune genes. All samples, both lesions and non-infiltrated areas, displayed very strong correlations ($P < 0.0001$) between transcription levels for PMCV, CD83, sIgM, TCR and MHC class II, respectively (Fig. 4a). Correlation testing of lesions alone yielded a higher degree of differentiation (Fig. 4b). The only immune gene that significantly correlated with PMCV was CD83 ($P < 0.01$). In addition, MHC class II transcription correlated with TCR ($P < 0.001$), sIgM ($P < 0.05$) and CD83 ($P < 0.05$). Finally, the transcript levels of sIgM and TCR were correlated ($P < 0.005$).

Discussion

The present study investigated the myocardial lesions characteristic of experimentally induced CMS in Atlantic salmon. Using combined laser microdissection and RT-qPCR, lesions and adjacent, apparently normal tissues were analysed for the presence of PMCV and PRV, viral pathogens associated with the cardiac diseases CMS and HSMI, respectively. PMCV transcripts were detected in all analysed lesions, but in only one corresponding normal tissue area. The detection of

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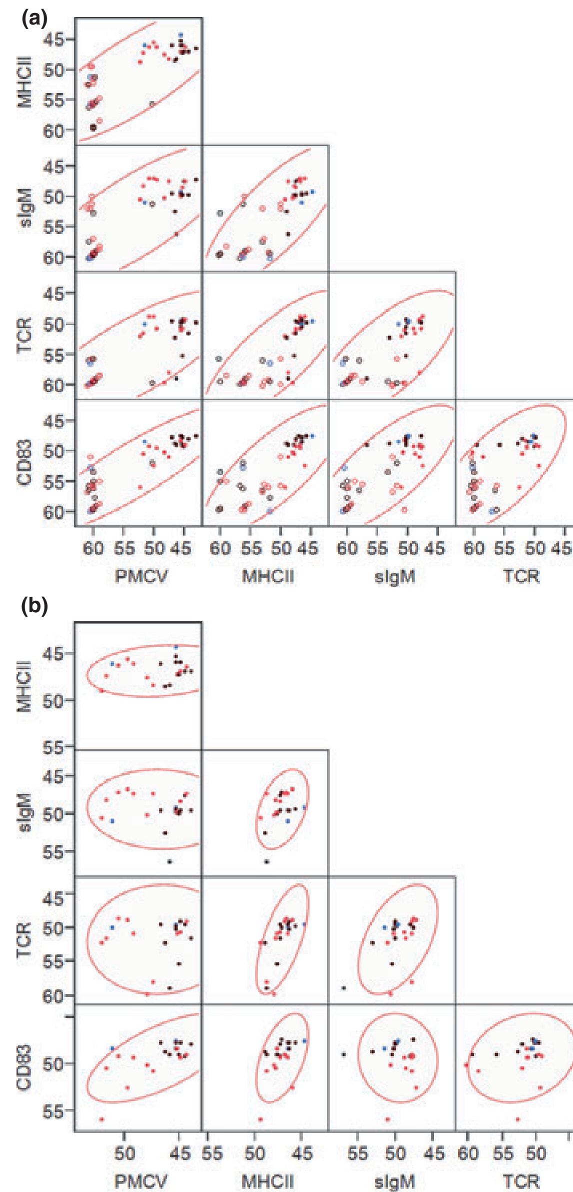

Figure 4 Pairwise correlation of transcript levels of piscine myocarditis virus (PMCV) and immune genes from laser capture microdissected samples. Each point represents a single microdissected sample; fish 1 (red), fish 2 (black) and fish 3 (blue). The red circles depict statistical 95% density ellipses. Axis values represent area-normalised Ct values. The table shows Kendall tau correlation values including respective *P*-values. (a) Correlation analysis including samples from both cardiomyopathy syndrome (CMS) myocardial lesions (filled dots) and normal tissues (open dots). (b) Correlation analysis of samples from myocardial lesions.

PMCV as late as 30 and 33 weeks post-challenge clearly shows that the PMCV genome persists in lesions despite massive infiltration of leucocytes. In contrast, PRV was not detected in lesions or normal tissues from the spongy myocardium, despite being present in total RNA isolated from the heart ventricle (Lovoll *et al.* 2010). This difference in virus distribution is consistent with PMCV as the aetiological agent of CMS (Haugland *et al.* 2011), and the absence of PRV in lesions typical for CMS indicates that PRV is not important for lesion development or persistence. This is further supported by an experimental challenge, showing that CMS lesions can develop in the absence of PRV (Haugland *et al.* 2011). Furthermore, it is well known that fish can contain high levels of PRV without showing histopathological changes typical of HSMI (Palacios *et al.* 2010). The lack of PRV within the investigated areas of spongy myocardium suggests that PRV is present elsewhere, for example in the compact myocardium or the epicardium. LCM in combination with RT-qPCR might be suitable tools for the identification of PRV positive cells in PRV positive but otherwise healthy Atlantic salmon.

The combined results from the immunohistochemical and RT-qPCR analyses demonstrate that

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several important leucocyte populations are present in the myocardial lesions. The significantly higher levels of TCR transcripts detected by RT-qPCR and the demonstration of $CD3^+$ cells by IHC both indicate that T cells are abundantly present in lesions, but not in apparently normal adjacent tissues. The findings of significantly elevated levels of sIgM transcripts (RT-qPCR) and the presence of IgM^+ cells (IHC) demonstrate increased numbers of cells of the B-cell lineage in the lesions. While both macrophages and NK cells have surface IgM Fc receptors, allowing binding of humoral IgM, the sIgM transcript data (RT-qPCR) directly demonstrates the presence of IgM-producing cells (plasmablasts and plasma cells). Similar local antibody production has been demonstrated in previous studies of virus-infected peripheral tissues in Atlantic halibut (Grove *et al.* 2006).

The level of MHC class II transcripts (RT-qPCR) was significantly higher in lesions than in normal tissues, an observation that was supported by immunohistochemical staining of MHC class II epitopes. As MHC class II is expressed by antigen-presenting cells (APC), the data indicate the local presence of cell types such as monocytes/macrophages, B cells (Vallejo, Miller & Clem 1992) and possibly granulocytes (Cuesta, Angeles & Meseguer 2006). Transcript levels of CD83, interpreted previously as representing activated macrophages (Donate *et al.* 2007), were also significantly increased in the myocardial lesions. As transcript levels of MHC class II and CD83 were strongly correlated, these data suggest that many of the indicated APCs are activated macrophages.

The relatively low levels of $sIgT_B$ transcription detected in some lesions combined with the almost complete absence of $sIgT_B$ transcription in normal tissue suggest some local production of this antibody within inflamed tissue. Immunoglobulin T is an antibody class specific to teleost fish, which has been recently linked to mucosal immunity (Zhang *et al.* 2010). However, other studies indicate that IgT transcription may also be important in systemic lymphoid tissues (Tadiso, Lie & Hordvik 2011). Thus, the current identification of IgT transcription in inflamed tissues may add to a more detailed understanding of the functions of IgT in teleost immunity.

Immunohistochemistry for PCNA demonstrated an increased number of proliferating cells and/or cells undergoing DNA repair, in, or close to, the myocardial lesions. The predominant morphology of $PCNA^+$ cells was consistent with that of cardiac myocytes. Endothelial-specific labelling revealed that endothelial cells were not particularly abundant. Negative van Gieson staining likewise excluded increased levels of fibroblasts within the lesions.

The finding that T cells, B cells and macrophages are abundantly present in CMS-related cellular infiltrates is in accordance with the situation in murine myocarditis induced by cytomegalovirus (Lenzo *et al.* 2002) or following myosin immunization (Pummerer *et al.* 1991; Afanasyeva *et al.* 2004). The strong correlation between transcript levels of PMCV and immune genes shows that leucocytes infiltrate cardiac tissues in response to viral infection (Fig. 4a). The correlation between the various immune cell types in the myocardial lesions (Fig. 4b) further indicates coordinated activity of leucocytes at the site of viral infection. While sIgM, TCR and MHC class II were mutually correlated with the infiltrates, only MHC class II showed correlation with CD83, which in turn was the only immune gene correlated with the PMCV viral levels. This hierarchy of correlations may suggest that activated ($CD83^+$) macrophages ($MHCII^+$) play a coordinating role in the developing lesions. In mammals, chronic infections may lead to *de novo* formation of local tertiary lymphoid microstructures resembling germinal centres (GC) (Carragher, Rangel-Moreno & Randall 2008). Teleost fish lack the advanced structure and function of GC (Bassity & Clark 2012), so a hypothetical tertiary lymphoid tissue in fish may be histologically primitive and hence hard to identify. While the observed cellular immune activity is most likely crucial in combating the PMCV infection, it may also have detrimental side effects by inducing damage within the infiltrated tissue. From mammalian models, it has been understood that the immune response to viral infection can be an important factor in the development of myocardial lesions by collateral damage inflicted by innate and adaptive immune effectors, but also through the development of autoimmunity (e.g. myocyte antigens; Blauwet & Cooper 2010). As such, typical CMS lesions may arise as a consequence of autoimmunity. However, the fact that PMCV was abundantly present in all examined myocardial lesions as late as 33 weeks post-challenge suggests that the viral infection remains ongoing and that viral lytic effects may

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be an important cause of the histopathological changes observed at this time of sampling.

In conclusion, we have demonstrated that PMCV is abundantly present in myocardial lesions characteristic of CMS, but is generally not present in adjacent normal tissues. The results confirm the association between PMCV and CMS. Despite the presence of PRV in the ventricles, PRV was not detected in any of the spongy myocardium tissues investigated. This indicates that PRV is not associated with the development or persistence of CMS lesions. We have further demonstrated that the cellular infiltrate is abundant in T cells, cells of the B-cell lineage and MHC class II^+ and $CD83^+$ cells, likely of macrophage origin.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Laser capture microdissected samples, number and size (nm^2). Corresponding areas (n = 22) consist of myocardial lesions and closely associated apparently normal tissues (control).

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