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Role of a macrophage receptor with collagenous structure (MARCO) in regulating monocyte/macrophage functions in ayu, *Plecoglossus altivelis*

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ABSTRACT

Macrophage receptor with collagenous structure (MARCO) plays essential roles in phagocytic cell-mediated innate immune responses. However, studies regarding MARCO, especially its functions, are limited in teleost species. In this study, we identified a MARCO molecule (PaMARCO) from ayu (*Plecoglossus altivelis*). PaMARCO shared conserved functional domains with its mammalian counterparts. Sequence analysis showed that PaMARCO was most closely related to its rainbow trout (*Oncorhynchus mykiss*) counterpart. PaMARCO expression was upregulated in all tested immune tissues and monocytes/macrophages (MO/MΦ) upon *Vibrio anguillarum* infection, and blocking its function significantly decreased the immune responses of MO/MΦ during infection. PaMARCO could bind to the tested gram-positive and -negative bacteria in a Ca^{2+}-dependent manner *in vitro*. Furthermore, the phagocytosis and bacterial killing activities of MO/MΦ were significantly decreased upon PaMARCO blockade using anti-PaMARCO IgG. PaMARCO was also involved in the polarization processes of ayu MO/MΦ. The upregulated expression of representative cytokines in LPS-induced M1 type (TNF-α, IL-1β) or cAMP-induced M2 type (TGF-β, IL-10) were inhibited in the anti-PaMARCO IgG-treated group, indicating that PaMARCO may be involved in the regulation of both inflammation priming and inflammation resolution of MO/MΦ. In conclusion, our results implicate that PaMARCO has essential regulatory roles for bacterial binding, clearance, and the polarization processes of ayu MO/MΦ.

1. Introduction

The innate immune responses are of prime importance for the immediate recognition and elimination of invading microorganisms. These responses are initiated by the recognition of pathogen-associated molecular patterns (PAMPs) through a limited number of germline-encoded pattern-recognition receptors (PRRs) [1]. To date, many PRRs have been identified and well-studied, especially in mammals, including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), cytosolic viral DNA sensors, the scavenger receptors (SRs), and others [2–6].

The macrophage receptor with collagenous structure (MARCO), which belongs to the class A scavenger receptor family (SR-A), is a nonopsonic phagocytic receptor mainly expressed by macrophages, dendritic cells, and certain endothelial cells [7–9]. MARCO was firstly isolated from the spleen of mice, and its molecular weight was 210 kDa, which was composed of three 52 kDa monomers [10]. Structurally, MARCO contains a short intracellular domain, a transmembrane domain, and a large extracellular part comprised of a spacer domain, the long collagenous domain, and the C-terminal cysteine-rich domain (SRCR) [7,10]. The SRCR is the typical domain of the SR-A family members, and mutagenesis studies with human MARCO revealed that the SRCR domain was essential for ligand binding and the subsequent immunological activities [11,12]. Similar to the other members of the SR-A, MARCO is involved in endocytosis, cellular migration, adhesion, phagocytosis, and antigen presentation in macrophages and dendritic cells [13,14]. Additionally, MARCO has been shown to bind oxidized lipids, unopsonized particles, a number of microbial components, and intact gram-positive and -negative organisms, and their nonredundant role in host defense has been established in a variety of bacterial infection macrophage models [15–18].

Recent studies have reported that MARCO also plays important roles in regulating macrophage polarization. At least two major phenotypes of macrophages, the classically type (M1) and the alternatively type (M2), are functionally polarized in response to pathogen infection and host mediators. The M1 type is mainly involved in pro-inflammatory responses and produces inducible nitric oxide synthase (iNOS) as well

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Table 1
Oligonucleotide primers used in this work.

Primers	Gene	Accession number	Nucleotide sequence (5′–3′)	Amplicon size (bp)
MARCO-c F	*PaMARCO*	MF804978	GGAATTCAYGGACACATTTGTGGATGG	1350
MARCO-c R			CAAGCTTTTAGATACATGTAACCCCAGC	
MARCO-p F	*PaMARCO*	MF804978	CGGATCCGTGCGCATTGTGGGTG	291
MARCO-p R			CAAGCTTTTAGATACATGTAACCCCAGC	
MARCO-t F	*PaMARCO*	MF804978	TGGTGAGAAGGGACAACTGGGCAAG	228
MARCO-t R			GCTTCACCTTTGTCTCCTTTTTCTC	
TNF-α-t F	*PaTNF-α*	JP740414	ACATGGGAGCTGTGTTCCTC	115
TNF-α-t R			GCAAACACACCGAAAAAGGT	
IL-1β-t F	*PaIL-1β*	HF543937	TACCGGTTGGTACATCAGCA	104
IL-1β-t R			TGACGGTAAAGTTGGTGCAA	
IL-10-t F	*PaIL-10*	JP758157	TGCTGGTGGTGCTGTTTATGTGT	73
IL-10-t R			AAGGAGCAGCAGCGGTCAGAA	
TGF-β-t F	*PaTGF-β*	JP742920	CTGGAATGCCGAGAACAAAT	88
TGF-β-t R			GATCCAGAACCTGAGGGACA	
18S rRNA-t F	*Pa18S rRNA*	FN646593	GAATGTCTGCCCTATCAACT	103
18S rRNA-t R			GATGTGGTAGCCGTTTCT	

Table 2
MARCO and SR-A sequences used in this study.

Accession number	Species		Protein
	Latin name	English name	
MF804978	*Plecoglossus altivelis*	ayu	MARCO
XM_012858122	*Fundulus heteroclitus*	mummichog	MARCO
XM_007575260	*Poecilia formosa*	Amazon molly	MARCO
XM_014173984	*Salmo salar*	Atlantic salmon	MARCO
XM_005813507	*Xiphophorus maculatus*	southern platyfish	MARCO
KJ955494	*Danio rerio*	zebrafish	MARCO
KX452014	*Oncorhynchus mykiss*	rainbow trout	MARCO
XM_007261049	*Astyanax mexicanus*	Mexican tetra	MARCO
NM_001303345	*Larimichthys crocea*	large yellow croaker	MARCO
XM_010880220	*Esox lucius*	northern pike	MARCO
XM_020461941	*Oncorhynchus kisutch*	coho salmon	MARCO
XM_018696612	*Lates calcarifer*	barramundi perch	MARCO
XM_020606488	*Monopterus albus*	swamp eel	MARCO
XM_017469237	*Ictalurus punctatus*	channel catfish	MARCO
XM_017692227	*Pygocentrus nattereri*	red-bellied piranha	MARCO
NM_006770	*Homo sapiens*	human	MARCO
U18424	*Mus musculus*	mouse	MARCO
XM_004550559	*Maylandia zebra*	zebra mbuna	SR-A
XM_005931531	*Haplochromis burtoni*	Burton's mouthbrooder	SR-A
XM_005475068	*Oreochromis niloticus*	Nile tilapia	SR-A
XM_008284982	*Stegastes partitus*	bicolor damselfish	SR-A
NM_001303357	*Larimichthys crocea*	large yellow croaker	SR-A
XM_020639946	*Labrus bergylta*	ballan wrasse	SR-A

as inflammatory cytokines like TNF-α and IL-1β. The M2 type play essential roles in resolving inflammation by producing arginase and anti-inflammatory mediators like IL-10 and TGF-β [19]. A study conducted on alveolar macrophages showed that MARCO acts as an initial signaling receptor for asbestos and polarizes macrophages to a profibrotic M2 phenotype [20]. Consistently, the transcriptome analysis of IL-10-stimulated (M2c) macrophages showed a specific expression of MARCO, and MARCO was also involved in LPS- and lipoteichoic acid (LTA)-induced immune tolerance [21,22]. However, *in vitro* cell study also showed that MARCO is highly expressed in M1 type macrophages at both the RNA and protein level, and MARCO contributes to efficient innate virus recognition of macrophages, leading to strong proinflammatory responses [23]. Hence, MARCO may have regulatory roles on both classically and alternatively activated macrophage subsets, depending on the source of macrophage and the pathogens present.

In contrast to the in-depth research of MARCO in mammals, knowledge about this molecule in teleost fish is rather limited. For now, the MARCO homologs have been identified in large yellow croaker (*Larimichthys crocea*), zebrafish (*Danio rerio*), and rainbow trout (*Oncorhynchus mykiss*) [24–26]. The gene expression patterns of MARCO in healthy and pathogen-infected tissues or monocytes/macrophages (MO/MΦ) have been clarified in all these three teleosts. Additionally, the ligand binding activity of MARCO has been analyzed in rainbow trout [26] and the roles of MARCO in phagocytosis of zebrafish MO/MΦ, as well as the induced inflammatory responses, have been elucidated [25]. Hence, a systematic study of the regulatory role of MARCO on the function of MO/MΦ in teleosts, including bacterial and ligand binding, phagocytosis, bacterial killing, and MO/MΦ polarization needed to be clarified.

Ayu (*Plecoglossus altivelis*) is an economically important fish that is widely cultured in East Asia. The development of ayu aquaculture has been challenged by bacterial and viral fish diseases that have caused production and animal welfare problems [27]. Given the important roles of macrophages in inflammatory responses, studying the regulatory mechanisms of ayu macrophages and investigating their possible roles in pathological processes are necessary. In the present study, a MARCO gene was cloned from ayu (PaMARCO). The mRNA expression patterns of PaMARCO were examined in various tissues under normal conditions and after *Vibrio anguillarum* challenge. The pathogen recognition activity and the regulatory roles of PaMARCO on the function and the polarization of ayu MO/MΦ were also assessed.

2. Materials and methods

2.1. Experimental fish

All experimental ayu used in the present study were purchased from a fishery in Ninghai County, Ningbo City, China. Healthy fish, weighing 40–50 g each, were kept in freshwater tanks at 20–22 °C with regular feeding as previously described [28]. The fish were acclimatized to laboratory conditions for two weeks before the experiments. All experiments were performed according to the Experimental Animal Management Law of China and approved by the Animal Ethics Committee of Ningbo University.

2.2. Molecular characterization of PaMARCO cDNA

The cDNA sequence of PaMARCO was retrieved from the transcriptome data of the ayu head kidney-derived MO/MΦ. Specific primers were designed to amplify the PaMARCO gene using RT-PCR (MARCO-c F/R, Table 1), followed by cloning and sequencing. Functional domains of PaMARCO were predicted by the SMART program (http://smart.embl-heidelberg.de/). The transmembrane helices were analyzed by TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). Multiple sequence alignment was generated by the