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Short communication

Zebrafish fatty acids receptor Gpr84 enhances macrophage phagocytosis

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ABSTRACT

GPR84 was identified as a receptor for medium-chain fatty acids with carbon chain lengths of 9–14. It has previously been reported that lipopolysaccharide (LPS) induces significantly up-regulation of zebrafish *gpr84*, and zebrafish *gpr84* overexpression markedly increased the LPS-stimulated production of the cytokine *IL-12*. Here we expanded on these studies to further investigate the roles of zebrafish Gpr84 in immune reaction. Flow cytometric assay was used to assess the effects of zebrafish Gpr84 on the phagocytosis of bacteria by macrophages. It was found that overexpression of zebrafish *gpr84* significantly increased both the phagocytic ability (PA) and phagocytic index (PI) values of the macrophages engulfing the bacteria, suggesting that zebrafish Gpr84 was able to promote the phagocytosis of bacteria by the macrophages. The data proves the direct effect of Gpr84 in immune reaction.

GPR84 belongs to the G protein-coupled receptor family, and it was first identified from human peripheral blood neutrophils [1,2]. GPR84 is now considered to be a member of receptor for medium chain fatty acid (MCFA) with carbon chain lengths of 9–14 [3]. GPR84 is mainly expressed in bone marrow, spleen, lung, lymph nodes, thymus and peripheral blood leukocytes of mice and is indicated to function as an immune cell-specific receptor for free fatty acids (FFA) [2,4]. The GPR84 activation in macrophages amplifies LPS-stimulated IL-12 p40 production [3], and a study using *Gpr84*-deficient mice also revealed a role of *Gpr84* in the regulation of *IL-4* gene expression in activated T cells [5]. Furthermore, GPR84 agonist can upregulate the Akt, ERK, and NF-κB signaling pathways, and increase bacterial adhesion and phagocytosis in murine macrophages [6]. In addition, GPR84-deficient mice exhibited smaller livers and increased triglyceride accumulation in response to FFA diet [7].

In fish, little information about Gpr84 is available. We have revealed that lipopolysaccharide (LPS) induces significantly up-regulation of zebrafish *gpr84*, and zebrafish *gpr84* overexpression markedly increased the LPS-stimulated production of the cytokine *IL-12* [8]. Here we expanded on these studies to further investigate the roles of zebrafish Gpr84 in immune reaction.

A murine macrophage-like cell line, RAW264.7 cell, was used to be transfected with the zebrafish *gpr84* ORF. The cells were cultured in DMEM medium (Gibco, Brazil) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Brazil), 100 IU/ml penicillin and 100 mg/ml streptomycin, in plastic cell culture flasks (Corning) at 37 °C in a humidified 5% CO2/95% air atmosphere.

Gene-specific primers with flanking restriction enzyme recognition sites (*Eco*RI and *Xho*I) were designed step over the intron to amplify the full length coding regions of Gpr84. Sense primer sequences were: 5′—CCGGAATTCATGGACACCACCGCTTTTGCA-3′. Anti-sense primer sequences were: 5′—CCGCTCGAGTCTTGAAGTAAACCAATGGGTTCTT-3′. PCR amplification was performed as reported previously (8). PCR products were subcloned into pcDNA3.1/V5-His vector for transformation. The plasmids were sequenced to ensure in frame subcloning. RAW264.7 cells were transfected with the pcDNA3.1/V5-His vector containing *gpr84* ORF using reagent of LF2000 (invitrogen) following the protocol provided. Thirty hours after transfection, undecanoic acid was added to the cells (final concentrations of 1 mM) for another 6 h.

Then flow cytometric analysis was preformed following the method of Sun et al. [9]. Aliquots of 300 μl FITC-labeled *Staphylococcus aureus* or *Escherichia coli* suspensions (1×10^8 cells/ml) were mixed with 300 μl of cells transfected with pcDNA3.1/V5-His vector containing the zebrafish-gpr84 ORF or the pcDNA3.1/V5-His vector alone. The mixtures were incubated at 25 °C under dark for 1 h. The phagocytosis process was terminated by addition of 600 μl ice-cold PBS (pH7.4), immediately followed by centrifugation at 200*g* at 4 °C for 5 min to separate the macrophages from non-phagocytosed bacteria. The cell pellets were re-suspended in PBS (pH7.4), and the fluorescence of the extracellular bacteria was quenched by adding 1 μl ice-cold trypan blue (0.4% in PBS) to each cell suspension. Immediately, a volume of 5 μl of the macrophage suspensions was sampled to make smears for examination by Leica confocal microscopy (TCS-SP8, Germany). Also, another 600 μl of the samples were mixed gently and analyzed in FC500

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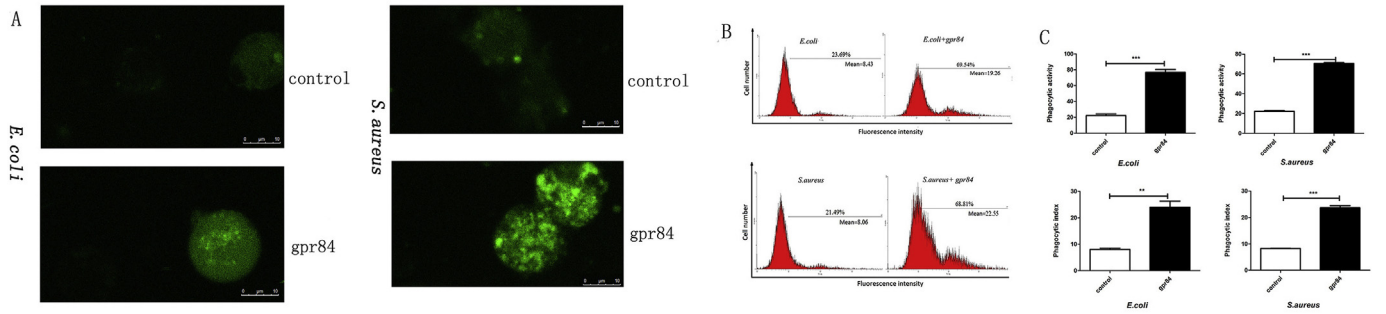

Fig. 1. Effects of zebrafish Gpr84 on the phagocytosis of macrophages. (A) FITC-labeled microbes and macrophages transfected with or without zebrafish *gpr84* ORF were mixed and incubated together. Phagocytosis was observed under Leica confocal microscopy (TCS-SP8, Germany). (B): The histogram of flow cytometric analyses of the macrophages phagocytosing *S. aureus* and *E. coli*. Data show analyses of 10000 events. The marker represented phagocytosis part of cellular population, and the PA (the percentage of the macrophages with one or more engulfed bacteria within the total macrophage population) and PI (mean fluorescence intensity) values were shown near the marker. (C): The statistics analysis of the PA and PI values of macrophages engulfing *E. coli* and *S. Aureus*. Vertical bars represent the mean ± SD (n = 3). Significant differences across control are indicated by asterisks (*p < 0.05, * *p < 0.01, ***p < 0.001).

MPL (Beckman) flow cytometer. The phagocytic ability (PA) was defined as percentage of the macrophages with one or more engulfed bacteria within the total macrophage population, and the phagocytic index (PI) as the mean fluorescence intensity of the cells. Statistical analyses were performed using the GraphPad Prism 5. The significance of difference was determined by two-way ANOVA. All the data were expressed as the mean ± SEM (n = 3). Difference at $p < 0.05$ was considered statistically significant.

Phagocytosis was observed at 60 min after mixing macrophages with microbes (Fig. 1A). The phagocytosis ability (PA) and phagocytosis index (PI) values of the macrophages engulfing the bacteria measured in flow cytometer were shown in histograms (Fig. 1B). Statistical analyses revealed that both the PA and PI values in zebrafish *gpr84*-overexpression macrophages engulfing *E. coli* were significantly increased compared with those of the cells transfected with pcDNA3.1/V5-His vector alone (Fig. 1C). Similarly, both the PA and PI values in zebrafish *gpr84*-overexpression macrophages engulfing *S. aureus* were also markedly elevated compared with those of the cells transfected with pcDNA3.1/V5-His vector alone (Fig. 1C). Collectively, these suggested that zebrafish Gpr84 was able to promote the phagocytosis of bacteria by the macrophages. The study expands our understanding of the role of zebrafish Gpr84 in immune reaction.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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