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The expression of two CD83 homologs in macrophage subpopulations isolated from brain and kidney of ginbuna crucian carp

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Abstract

There are numerous fish diseases that affect central nervous system. However, few studies have investigated at the immune cells and immunological responses of fish brain. Meanwhile, microglial cells as the brain's first line of defense play a vital role in neuroimmunology. Furthermore, CD83 is a co-stimulatory protein that regulates immunological responses and the activation of dendritic cells and macrophages. While CD83 expression has been linked to the initial activation of microglia in a variety of diseases scenarios in mammals, its role in teleost microglial biology remains unclear. In a recent investigation, we discovered that ginbuna crucian carp (Gb) contains two CD83 homologs (GbCD83 and GbCD83-L). In this study, we used modified mouse-based macrophage culture procedures from the brain and kidney to discover that GbCD83-L is highly expressed by brain microglia-like cells and kidney-resident macrophages (KRMs) at both protein and gene levels. Interestingly, *GbCD83-L* was considerably elevated in microglia-like cells and KRMs after 24 hours of lipopolysaccharide stimulation. These findings provide the first evidence of CD83 as a potential marker for active microglia and KRMs in teleosts, positioning it as a crucial regulator in fish neuroimmunology and a candidate for immunomodulatory applications in aquaculture.

1 Introduction

Macrophages are myeloid cells in innate immunity that engulf and digest foreign substances, dead cells, and debris, as well as attract other macrophages in response to inflammatory triggers [1]. Mammals possess two types of macrophages: embryonic and monocyte-derived macrophages. Furthermore, they are distributed strategically throughout various tissues and classified into different subpopulations based on their anatomical location and functional characteristics, such as osteoclasts (bone), alveolar macrophages (lung), histiocytes (interstitial connective tissue), Kupffer cells (liver), kidney resident macrophages, and microglia (brain). Teleost fish macrophages, like their human counterparts, assist in regulating homeostasis and host immunological responses [2], and tissue-resident macrophages have also been conserved in fish. Furthermore, zebrafish have been shown to have tissue-resident macrophages, including myeloid-derived microglia [3]. Microglia are derived from the embryonic yolk sac, whereas peripheral macrophages come from hematopoietic stem cells [4]. Microglial cells are macrophages that reside in the brain and serve as first line cells in the central nervous system (CNS). Microglia can be discovered by the purinergic receptor (P2RY12) and transmembrane protein 119 (TMEM119), which were previously identified as the most specific general microglia markers [5]. Microglia serve primary functions such as monitoring the environment and initiating an inflammatory response when a danger signal is perceived in the brain. Microglia are involved in a variety of activities in the mammalian nervous system, including synaptic plasticity and brain homeostasis [6]. Extensive study has been undertaken on macrophages in the spleen and kidney of teleosts, however the findings on microglia remain contentious. Embryonic and larval zebrafish have been widely used as models for investigating the location and function of microglial cells during brain regeneration. These microglial cells have been shown to play important roles in neuroinflammatory and oxidative stress processes during zebrafish brain regeneration [7].

In mammals, CD83 is an immunoglobulin protein and co-stimulatory molecule that is generally known as a surface marker of mature dendritic cells, monocytes, and macrophages in humans [8], making it a fascinating possibility for macrophage biological manipulation. Macrophages express CD83 as a rapid and early response to inflammation [9]; also, deleting CD83 in these cells results in an impaired pro-resolving characteristic in mice [10]. Furthermore, CD83 expression is predominantly restricted to homeostatic murine microglia and border-associated macrophages in CNS [10] [11]. The presence of CD83 shows a subset of human microglia that have been pre-activated, similar to microglia associated with diseases [12]. In teleosts, CD83 expression has been detected largely in dendritic cells [13], macrophages [14], monocytes [15], and monocyte-derived dendritic cells [15], but not in microglia.

Meanwhile, CD83 expression in murine microglia has been shown to act as a regulating mechanism for microglial activation during neuroinflammation [16]. This shows that CD83 expression may also govern teleost microglial cells, but its practical importance in microglial biology is uncertain.

In the previous study, we discovered two CD83 homologs, GbCD83 and GbCD83-L, in clonal ginbuna crucian carp (Gb) (*Carassius auratus langsdorfii*) (S3N clone), an effective model for examining adaptive immune activation [15] . The current work examines the expression of these CD83 homologs on various subgroups of macrophages from the brain and kidney, specifically microglial-like cells and kidney-resident macrophages (KRMs). These findings give the first evidence of CD83 expression in teleost microglia while also examining the shape of microglial cells in contrast to KRMs. Furthermore, CD83 has been shown to boost protective immunity in turbot using a DNA vaccine [17] and its activity can be altered by viral and bacterial infections in sea bass and grouper [18,19]. In this study, we examined the expression of CD83 homologs following lipopolysaccharide (LPS) stimulation to confirm CD83 function in microglia and KRMs. As a result, this discovery may pave the way for more research into CD83's role as a regulator of both microglia and innate immune cells during infection in fish. It could help with the development of a vaccination adjuvant in aquaculture employing CD83.

2 Materials and Methods

2.1 Fish

The study used clonal triploid S3N Gb (*C. auratus langsdorfii*). At Kyushu University, the fish were maintained in tanks with a constant supply of recirculating freshwater set at 25°C. They were given commercial pellets produced by Nippon Formula Feed Manufacturing Co., Ltd. in Japan twice a day. Brain and kidney samples were collected from one-year-old fish weighing between 20 and 30 g. The trials followed the guidelines of the Animal Experiments Committee at Kyushu University.

2.2 Surgical procedure

The fish were sedated with 2-methylquinoline dissolved in 5% ethanol until their swimming became unsteady, after which their bodies were cleaned with 70% ethanol cotton. Blood was collected from the fish's caudal vein using a syringe before dissecting the fish to obtain the kidney, and the skull was opened to extract the brain. The brain was then submerged in ice-cold DMEM/F12 (Gibco, USA), while the kidney was transferred to ice-cold L-15 (Gibco, USA).

2.2 Culturing of microglial cells and kidney-resident macrophage

The optimized approach was employed to isolate microglial cells (brain macrophages) and KRMs without enzymatic dissociation and based on their plastic adherent capabilities, as depicted in Figure 1. The brains were chopped into smaller pieces using scissors and mechanically dissociated by gently

triturating them with three Pasteur flame-polished pipettes. The aperture sizes of the first, second, and third pipets were 0.75-0.8 cm, 0.5-0.55 cm, and 0.2-0.25 cm, respectively. Perform centrifugation at 500 g for 10 min at 4°C. The supernatant was removed and reconstituted with DMEM/F12 medium with 10% Fetal Bovine Serum (FBS). The cells were mixed with 6 ml of Percoll 70% (P70) and then layered with 3 ml of Percoll 50% (P50) and 35% (P35). Following that, 2 ml of Hanks' Balanced Salt Solution (HBSS) (Gibco, USA) was added to the top layer, and the mixture was centrifuged at 300 g for 29 minutes at 18°C. Microglial cells and other leukocytes were isolated from the interfaces of P70/P50 and P50/P35. Myelin was isolated from brain leukocytes at the P35/HBSS (Fig. 1). Leukocytes were grown in DMEM/F12 medium supplemented with 4mM $NaHCO_3$, 15mM HEPES, 50 units of penicillin-streptomycin, and 10% fetal bovine serum (Biowest, France). The quantity and vitality of leukocytes were determined by counting viable cells after trypan blue staining. Cell viability was around 95%, with a cell density of 5×10^6 cells/ml supplied to 24-well plates. The plates were placed in an incubator set to 25°C for 6 hours. The cells were then washed with DMEM/F12 to remove nonadherent cells. Adherent cells were grown in the cell culture medium at 25°C and 5% CO_2 for 6 days.

To separate the macrophages in the kidneys, they were homogenized on a stainless-steel sieve with a syringe plunger. Leukocytes were separated using a Percoll density gradient with a density of 1.08 g/mL. The cells were then rinsed twice with L-15 media (Gibco, USA), and the concentration and vitality of leukocytes were determined by counting viable cells with trypan blue. Cell viability was almost 95%. Leukocytes were cultured in L-15 media at a concentration of 5×10^6 cells/ml. The culture medium included 50 units of penicillin and streptomycin, 10% fetal bovine serum (Biowest, France), 12.5 mM HEPES, 2-mercaptoethanol, 7.5% $NaHCO_3$, and 7% Gb serum. The cells were incubated in 24-well plates. The plates were incubated at 25°C for 5 hours. The cells were then rinsed with L-15 to eliminate any non-adherent cells. The adherent cells were grown in cell culture medium at 25°C and 5% CO_2 for 6 days (Fig. 1).

2.3 Quantitative measurement of gene expression

Total RNA was extracted from adherent, nonadherent, and 6-day-cultured cells using TRIzol reagent (Invitrogen, USA). The first-strand cDNA for gene expression analysis was generated using M-MLV reverse transcriptase (Nippon Gene, Japan) per the manufacturer's instructions. Total RNA (200 ng) extracted from cells was utilized to synthesize cDNA. Quantitative PCR (qPCR) was used to identify microglial genes (purinergic receptor (*P2RY12*), spalt-like transcription factor 1 (*SALL-1*), transmembrane protein 119 (*TMEM119*), and KRMs genes (colony stimulating factor 1 receptor (*CSF-1R*) and Interleukin-1b (*IL-1b*)), in both adherent and nonadherent cells. In addition, *GbCD83* and *GbCD83-L* are also quantitated in cells that had been cultured for 6 days. To prevent the amplification of genomic DNA, distinct forward and reverse primers were designed for various exons. The qPCR experiment was carried out on a Stratagene Mx 3000P machine, following the manufacturer's instructions for the Quantitect SYBR Green PCR kit. The reaction consists of SYBR buffer, DEPC water, ROX, 0.3 µM primers, and 1 µl cDNA template. Follow the thermal procedure below: The experiment consisted of 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds, then back to 95°C for 15 seconds. To measure target genes, cDNA from Gb head kidney and brain were utilized as standard

samples, with *EF-1α* serving as an internal reference gene for standardization. The primers' effectiveness was evaluated using the dissociation curve (melting curve). All of them had a single peak of dissociation. Each sample was tested on three fish. The efficiency of CD83 and CD83-L primers is 99.6% and 99%, respectively. The qPCR primers are listed in Table 1. The fold difference relative to reference gene of genes were assessed using the relative standard curve method. The statistical significance of two samples was determined using a t-test with unequal variances. Significant values were defined as $p < 0.05$.

2.5 Antibodies

The mouse monoclonal antibody 4C4 (m4C4Ab), a gift from Prof. Peter Hitchcock of the University of Michigan, was used to detect microglial cells among the adherent cells of the Gb brain. It was first created utilizing protein extracts from the optic nerves of tilapia (*Oreochromis mossambicus*), specifically 12 days after damage. It is typically used to detect microglia in embryonic and larval zebrafish using immunofluorescence studies [20,21]. The microglial m4C4Ab has been found targeting the antigen zebrafish galectin 3 binding protein (Lgals3bp) [21]. The identification of microglia in Gb with m4C4Ab was confirmed by flow cytometry. Briefly, leukocytes were extracted from the brain, kidney, and gills using Percoll gradient centrifugation. Cells were treated with the primary antibody m4C4 at a dilution of 1:500 following to a previous study [21] for 1 hour, followed by the secondary antibody Alexa FluorTM 488 goat anti-mouse IgG (H+L) (Invitrogen, USA) at a dilution of 1:500. Simultaneously, leukocytes were treated with 1.0 μg/mL of isotype IgG antibody (MBL, Japan) as a control antibody. Finally, cells were run through the Attune NxT Acoustic Focusing Cytometer for flow cytometry analysis, which was repeated three times. Furthermore, our earlier research described the procedure of manufacturing rabbit anti-peptide CD83 polyclonal antibodies (GbCD83pAbs) for the aim of recognizing GbCD83 and GbCD83-L in Gb [15]. These antibodies were subsequently used to determine the expression of CD83 protein on brain and kidney cells.

2.6 Immunocytochemistry

Fresh adherent cells, nonadherent cells, and 6-day cultured cells were fixed on slides by cytospin centrifugation at 800 rpm for 2 minutes and then dried. The cells were exposed to a formaldehyde-acetone buffer solution at 4°C for 30 seconds. The cells were then neutralized using a 0.3% hydrogen peroxide solution for 30 minutes. The immunocytochemistry approach included labeling cells with the VECTASTAIN Elite ABC kit-peroxidase (HRP; Vector Laboratories, USA). The cells were treated with a normal blocking serum for 20 minutes. Following that, primary antibodies (m4C4Ab (1:500 dilution), GbCD83pAbs (1.0 μg/mL), and purified pre-immune rabbit IgG (1.0 μg/mL) were added. Next, a biotinylated secondary antibody and the VECTASTAIN Elite ABC reagent were added. The substrate used for color development was 3,3'-Diaminobenzidine Tetrahydrochloride. The stained samples were mounted in an EUKITT mounting medium (ORSAtec GmbH, Germany) and covered with coverslips. Cell samples were examined under a microscope. The studies were carried out in duplicate, and the proportion of positive cells was determined under a microscope.

2.7 Cellular Morphology

Adherent cells and 6-day cultivated cells were stained with Giemsa solution (Merck, Germany) to view and study their cellular morphology. As indicated in Section 2.6, the cells were spread onto slides and stained with Giemsa for 20 minutes at room temperature before being rinsed with water and examined under a microscope. The microscope was also used to collect adherent cells and cells that had developed for 4 to 6 days from both the brain and the kidney.

2.8 *In vitro GbCD83* homologs expression during LPS-induced stimulation

Microglia and KRMs were grown from the brain and kidney *in vitro*, as described in Section 2.2. On day 2 of growth, 1 µg/mL of lipopolysaccharide (LPS from E. coli 055: B5, Enzo) was introduced to cell wells (2×10^5 cells/well). The non-stimulated control samples were used as a calibrator. Total RNA was extracted using TRIzol reagent. M-MLV reverse transcriptase was used to produce first-strand cDNA for gene expression investigation. *GbCD83-L* transcription was measured using qPCR at 6-, 12-, and 24-hours post-challenge. The primers and real-time PCR conditions were exactly as reported in section 2.3. The fold difference in gene expression levels between the target gene and calibrator in cells were assessed using the relative standard curve method. The control was set at 1 (baseline). The statistical significance of two samples was determined using a t-test with unequal variances. Significant values were defined as $p < 0.05$.

3 Results

3.1. Gene expression of microglia and macrophages on fresh adherent and nonadherent cells

Brain adherent cells showed significant expressions of *P2RY12*, *SALL-1*, *TMEM119* compared to brain nonadherent cells (Fig.2A). Besides, kidney adherent cells highly express *CSF-1R* and *IL-1b* compared to kidney nonadherent cells (Fig.2B). *CSF-1R* has been illustrated as a specific marker for monocytes/macrophages in goldfish, and macrophage-colony stimulating factor (*M-CSF*) signaling through *CSF-1R* to promote the differentiation of macrophages [22]. In addition, SALL-1 was used to establish microglia identity [23], P2RY12, TMEM119 have been widely employed as specific markers to detect microglial cells in mammals and zebrafish [20,24]. As a result, mRNA expression levels illustrated that brain adherent cells are enriched microglial-like cells, and kidney adherent cells abundantly include KRMs.

3.2. Cell morphology

Adherent cells from the brain and kidney had been examined over a 6-day culture period. Morphological study demonstrated that brain adherent cells initially exist as spherical cell monolayers (Fig. 3 A, D), but after 6 days, they gradually expanded in size and underwent a morphological change into ramified, bright-phrase cells (Fig. 3 B, C). Giemsa staining revealed circular mononuclear cells on

day 6 of culture (Fig. 3E). The aforementioned morphological characteristics have also been documented in microglia of goldfish and mouse [25,26]. Adherent microglial cells in goldfish were found to have a bright-phrase spherical shape, and these cells exhibited a variety of morphologies beginning on day 3, including round, ramified, and macrophage-like forms. Furthermore, mouse microglia were cultivated based on their adhesive properties, with a spherical morphology during the adherent stage, followed by a transition to a ramified morphology and a bright appearance. Morphological parameters indicate that the brain 6-day grown cells have a morphology similar to microglia.

The kidney cells stick uniformly to the plastic plate (Figure 3 F, I), and then display circular and elongated shapes, as well as bright patterns from day 3 to day 6 of their growth (Figure 4 G,H). Furthermore, Giemsa staining revealed a significant presence of vacuoles (Fig. 3 J) within renal cells, a characteristic of macrophages [27].

3.3. Detection of microglia-like cells from brain adherent cells by m4C4Ab

Microglia-like cells were detected by staining adherent cells isolated from the brain with m4C4Ab and goat anti-mouse IgG (biotin) (Abcam). The adherent cells (92±3%) exhibited positive signals (yellow color) (Fig. 4A,B), whereas nonadherent cells displayed negative signals (Fig.4C). In addition, the adhering cells were subjected to staining using pre-immune serum IgG (as a control antibody), yielding negative results (Fig.4 D). Flow cytometry was used to verify the specific binding of m4C4Ab to brain cells in Gb, as demonstrated in the supplementary material (Fig.S1). Microglial cells were distinguished from total brain leukocytes in the R2 gate based on their size and internal complexity, as observed by morphological analysis. The findings indicate that there were substantial signals from gate R2 exclusively observed in brain cells, but no signals were detected in the cell populations of the kidney and gills. All of these findings demonstrate that the brain adherent cells consist of an enhanced population of microglia-like cells.

3.4. Gene and protein expressions of GbCD83 homologs on cultured microglia-like cells and kidney-resident macrophages

Adherent cells obtained from the brain and kidney were grown for a duration of 6 days. Subsequently, these cells were utilized to assess the expressions of CD83 at both the gene and protein levels. The immunocytochemistry results indicate that 6-day microglia-like cells (Fig.5 A) and KRMs (Fig.6 A,B) exhibit positivity for GbCD83-LpAb at rates of 89±4% and 95±3%, respectively. Conversely,

microglia-like cells show a negative response to GbCD83pAb (Fig.5 B) and the control antibody (Fig.5 C). Additionally, KRMs were observed negatively to GbCD83pAb (Fig. 6 C,D) and the control antibody (Fig.6 E). As a result, both microglia-like cells and KRMs have a high expression of GbCD83-L protein. Simultaneously, the mRNA expression pattern demonstrated an upregulation of *GbCD83-L* in the 6-day microglia-like cells and KRMs shown in Fig.5 D and Fig.6 F, as compared to *GbCD83*.

3.5. GbCD83 homologs gene expression after LPS-stimulation *in vitro*

On the second day of culture, the addition of LPS (1 μg /mL) resulted in the stimulation of microglia-like cells and KRMs. Consequently, the expression of *GbCD83-L* gene in microglial-like cells was seen to be suppressed within the time frame of 12 hours following the challenge, followed by a notable upregulation after 24 hours (Fig.7A) In addition, this gene exhibits a significant increase at 12-hour period (Fig.7B).

4 Discussion

The use of plastic adhesion to isolate peripheral macrophages from the kidney or spleen has become established as a benchmark in mammalian macrophage research [28]. Glial cell primary cultures can be easily obtained from the brains of fetal or neonatal mice. Nevertheless, fish glial cell cultures are largely derived from the optic nerve or retina [29]. Only a few studies have documented microglial cell (brain macrophage) cultures from the brains such as puffer fish [30], goldfish [25], and Nile tilapia [31]. The enzymatic dissociation method has been widely used in the culture of microglial cells in animals. In our first attempts to separate microglia from Gb brain, we used a modified enzymatic dissociation method employing Collagenase A, a protocol used to isolate microglia in mice or humans [32]. We next incubated the isolated brain leukocytes with m4C4Ab and Propidium Iodide to determine microglial cells viability using flow cytometry. The percentage of live m4C4Ab positive cells dissociated by Collagenase A was much lower than that of cells untreated with the enzyme (data not shown). The enzyme was considered to have a negative impact on the viability of microglial cells, which has also been found in goldfish microglial cell culture [25]. Furthermore, previous investigations have shown that microglial cells and astrocytes coexist in mixed glial cultures in goldfish and puffer fish [25,30]. The use of these culture techniques would hamper the research of gene expression, because of astrocytes contamination. As a result, a nonenzymatic separation approach based on plastic adhesion was established for microglia monoculture in the absence of mixed astrocytes. The technology was developed by combining mouse microglia isolation protocols employing in vitro

adherent capabilities [26] and microglia culture in Nile tilapia [31] to acquire pure microglial cells from the complete brain cell population. The use of this methodology would aid in the understanding of fish microglial cells' functional roles *in vitro*. Furthermore, particular microglial genes (*P2RY12*, *SALL-1*, and *TMEM119*) were found to be significantly expressed in Gb brain adherent cells. Simultaneously, the m4C4 antibody, which binds specifically to microglia in teleosts, positively stained brain adherent cells rather than nonadherent cells. Furthermore, after 6 days of growth, the cells showed the ramified shape and central nucleus, which is consistent with descriptions of similar traits of microglial cells in mammals and goldfish [25] [26]. The results unequivocally showed that Gb brain adherent cells are microglia-like cells. In contrast, the morphology of KRMs changed to round, elongated, and heavily vacuolated after 6 days of growth. In addition, morphological observations revealed that Gb microglia-like cells tended to rest in the absence of activation circumstances, whereas KRMs had a significant level of mobility during the culture period (Video Supplementary Data). It is worth noting that in the presence of stimulation, we observed that microglia-like cells demonstrated increased motility and the formation of long branches (data not shown). The similar activities have been observed in microglia from both mice and humans [26].

CD83 is expressed in monocytes [15], macrophages [14], and dendritic cells [13] in teleosts. Furthermore, two CD83 homologs have been illustrated that exhibit distinct expression patterns during monocyte differentiation in Gb [15]: GbCD83 was discovered to be expressed in monocytes, but GbCD83-L was more highly expressed in monocyte-derived dendritic cells. It has been discovered that two CD83 homologs have separate evolutionary paths, resulting in different biological activities. Notably, prior research indicated that *GbCD83-L* was more abundant in the brain than other tissues, implying that *GbCD83-L* will be widely expressed throughout the CNS, rather than just in lymphoid organs where immune cells are located. In the present work, GbCD83-L was also shown to be substantially expressed in microglia-like cells in the brain and KRMs at both the gene and protein levels. The expression of CD83 on teleost microglia is not entirely known. However, a recent study found that murine microglia lacking in CD83 attract more harmful immune cells to the central nervous system, reducing the ability to resolve and worsening the diseases. CD83 thus plays an important function in controlling cellular activation and, as a result, neuroinflammation resolution in murine microglia [16]. It implies that CD83 expression on microglia may also play a role in regulating neuroinflammation in teleosts. As a result, detecting CD83 expression on Gb microglia-like cells would serve as a marker for identifying microglia cells in teleost brains. In order to further investigate of the immunological function of CD83, we examined the expression of *CD83-L* under stimulation

circumstances, and our data showed that the expression *GbCD83-L* increased in microglia-like cells and KRMs after LPS treatment, suggesting *GbCD83-L* may play a role in the remission of inflammation in macrophage subpopulations in the brain and kidney in Gb. Our findings could serve as a platform for future research into the involvement of CD83 in neuro-immunology in teleost. Previous research found that stimulating cultured sea bream macrophages with *Escherichia coli* LPS significantly boosted *CD83* mRNA expression [14]. Similarly, bacterial infection and poly (I:C) therapy caused a time-dependent increase in turbot *CD83* expression in the kidney [33]. Nonetheless, administering LPS to head kidney leukocytes *in vitro* resulted in a decrease in *CD83* mRNA levels in sea bass at both the 4-hour and 24-hour time intervals [18].

Our modified methodology for *in vitro* cultivation of microglia-like cells in Gb helped to determine differences in cellular morphology and behavior between two separate subpopulations of macrophages specifically between microglia-like cells (brain macrophages) and kidney-resident macrophages. The discovery of CD83 expression on microglia-like cells generated from the brain and KRMs in Gb, as well as the significant increase in *CD83-L* homologs following 24-hour LPS-induced stimulation, suggests that CD83 may serve as a potential marker of active microglia and KRMs in teleost. All findings would contribute to develop CD83 as an immunomodulator or vaccine adjuvant.

Declaration of Competing 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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Data Availability Statement

Data will be made available on request.

5 Figure legends

Figure 1. The procedure of isolation of microglia-like cells and kidney-resident macrophages from the brain and kidney of ginbuna crucian carp. The aperture sizes were 0.75-0.8 cm (#1), 0.5-0.55 cm (#2), and 0.2-0.25 cm (#3), respectively.
(*Created with BioRender.com*)

Figure 2. (A) The gene expression of *P2RY12, SALL-1, TMEM119* on adherent cells (Ad) and nonadherent cells (Non-ad) in brain. (B) The gene expression of *CSF-1R*, *IL-1b* on adherent cells (Ad) and nonadherent cells (Non-ad) in kidney. Normalized by housekeeping gene *EF-1α*. Results are the mean of three individual fish (n = 3); error bars indicate SD. An asterisk indicates a significant difference in gene expression between cell groups (* $p < 0.05$, ** $p < 0.01$).

Figure 3. (A–E) Microscopic images of brain cells through 6 days: (A) fresh isolated adherent cells, (B) cells cultured for 4 days, (C) cells cultured for 6 days, (D) fresh adherent cells (Giemsa stain), and (E) cells culture for 6 days (Giemsa stain). (F–J) Microscopic images of kidney cells through 6 days: (F) fresh isolated adherent cells, (G) cells cultured for 4 days, (H) cells cultured for 6 days, (I) fresh adherent cells (Giemsa-stain), and (J) cells culture for 6 days (Giemsa strain). Scale bar, 20 μm. Microscopic images (40× magnification).

Figure 4. The detection of microglial cells in Gb brain's adherent and nonadherent cells using m4C4Ab. (A–B) Fresh adherent cells stained by m4C4Ab; (C) Nonadherent cells stained by m4C4Ab; (D) adherent cells stained by pre-immune antiserum antibody (control antibody). Microscopic images (40× magnification). Scale bar, 20 μm.

Figure 5. The protein expression of GbCD83 homologs on 6-day cultured microglia-like cells (A-C): (A) Cells stained by CD83-LpAb, (B) cells stained with CD83pAb, (C) cells stained with stained with the pre-immune antiserum antibody. Microscopic images (40× magnification). Scale bar, 20 μm; (D) The gene expression of *GbCD83* and *GbCD83-L* on 6-day culture microglia-like. Normalized by housekeeping gene *EF-1α*. Results are the mean of three individual fish (n = 3); error bars indicate SD. An asterisk indicates a significant difference in gene expression between groups ($p < 0.05$).

Figure 6. The protein expression of GbCD83 homologs on 6-day cultured kidney-resident macrophages (A-E): (A, B) Cells stained by CD83-LpAb, (C, D) cells stained with CD83pAb, (E) cells stained with stained with the pre-immune antiserum antibody. Microscopic images (40× magnification). Scale bar, 20 μm; (F) The gene expression of *GbCD83* and *GbCD83-L* on 6-day culture cells. Normalized by housekeeping gene *EF-1α*. Results are the mean of three individual fish (n = 3); error bars indicate SD. An asterisk indicates a significant difference in gene expression between groups ($p < 0.05$).

Figure 7. The gene expression of *GbCD83-L* during LPS-induced stimulation in cultured microglia-like cells and kidney-resident macrophages. (A) *GbCD83-L* gene expression in cultured microglia-like cells from the brain at the period of 6,12,24 hours post LPS-challenge. (B) *GbCD83-L* gene expression in cultured KRMs at the period of 6,12,24 hours post LPS-challenge. Normalized by housekeeping gene *EF-1α*. Non-stimulated control was used as a calibrator, and set at 1. Results are the mean of three individual fish (n = 3); error bars indicate SD. Asterisks indicate a significant difference in gene expression between cell groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). NS, no significant.

Highlights

- Brain and kidney macrophages exhibit differences in morphology and cell behavior.
- *GbCD83-L* is highly expressed in both microglia-like cells and kidney-resident macrophages (KRMs).
- *GbCD83-L* expression is upregulated in microglia-like cells and KRMs following LPS stimulation.
- The expression profiles suggest its potential as a promising immune stimulator.