

Short Communication

Identification of the Fc α / μ R Isoform Specifically Expressed in the Kidney Tubules

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Abstract

Fc α / μ R is expressed not only in lymphoid, but also in non-lymphoid organs, including kidney. However, molecular and functional characteristics of Fc α / μ R, particularly expressed in non-lymphoid organs, have remained unclear. Here we identified an isoform of Fc α / μ R in the murine kidney on the C57BL/6J background. The kidney expressed only the isoform, which was not expressed in other lymphoid and non-lymphoid organs, and this isoform binds both IgA and IgM. Immunohistochemical analyses suggested that the Fc α / μ R isoform was expressed in the tubular epithelial cells, but not in the glomeruli. This was confirmed by flowcytometry analysis of isolated tubular epithelial cells and by RT-PCR analyses using the separately excised glomerular and tubular regions by the laser microdissection system. These results suggest that Fc α / μ R may not be involved in IgA deposition in glomerular mesangium cells in IgA nephropathy. Rather, it may play an important role in immunity in the renal tubular regions.

Keywords; Fc α / μ R, Fc receptor, IgM, IgA, IgA nephropathy

1. Introduction

Fc receptors (FcRs) for immunoglobulins mediate various immune responses upon binding to antibody-antigen immune complexes (Daeron, 1997) (Ravetch and Clynes, 1998). Their pivotal roles in immune responses have been extensively documented by analyses of mice lacking FcR genes for IgG (*Fc γ RI*, *Fc γ RII*, and *Fc γ RIII*) and IgE (*Fc ϵ RI* and *Fc ϵ RII*) (Ravetch and Bolland, 2001; Takai, 2002). In contrast to the FcRs for IgG and IgE, molecular and functional characteristics of both human and mouse FcR for IgM had long been undetermined. Moreover, although an Fc receptor for IgA (CD89) was identified in human (Maliszewski et al., 1990), mouse Fc receptor for IgA had not been identified. We previously identified an Fc receptor for both IgA and IgM, designated as Fc α/μ receptor (Fc α/μ R), in human and mouse (Shibuya and Honda, 2006; Shibuya et al., 2000). The *Fc α/μ R* gene is mapped to syntenic regions of mouse chromosome 1 (1F) and human chromosome 1 (1q32.3), near several other Fc receptors including Fc γ receptors I-III, the Fc ϵ receptor, and the polymeric immunoglobulin receptor (Kinet and Launay, 2000; Shimizu et al.,

2001), and encodes a type I transmembrane protein with single immunoglobulin (Ig)-like domain in the extracellular portion.

$Fc\alpha/\mu R$ is expressed not only in lymphoid, but also in non-lymphoid organs, including kidney (Sakamoto et al., 2001). However, molecular and functional characteristics of $Fc\alpha/\mu R$, particularly expressed in the non-lymphoid organs, have remained unclear. Because Fc receptor for IgA may be involved in variable human diseases (Monteiro and Van De Winkel, 2003), including IgA nephropathy (Moura et al., 2008), it is quite important to characterize the expression and function of $Fc\alpha/\mu R$.

Here, we show that murine kidney expresses only the isoform of $Fc\alpha/\mu R$, which is not expressed in other tissues, and examined the localization of the $Fc\alpha/\mu R$ isoform in the kidney.

2. Materials and Methods

2.1. Cells, mice and antibodies

BW5147 is a mouse thymoma cell line. BW5147 transfectants stably expressing Fc α / μ R isoforms were established, as described (Shibuya et al., 2000). C57BL/6J and BALB/c mice were purchased from Clea Japan, inc. (Tokyo, Japan) and maintained under the specific pathogen free (SPF) condition. The Fc α / μ R-specific monoclonal antibodies were established previously (Shibuya et al., 2000). Fluorescein isothiocyanate (FITC)- or Allophycocyanin (APC)-conjugated streptavidin, mouse IgM, IgA and anti-mouse CD13 were purchased from PharMingen (San Diego, CA).

2.2. 5' RACE

Total RNA was isolated from the kidney of mice by using ISOGEN[®] (Nippon Gene Co., Ltd, Tokyo, Japan). 5' RACE analysis were carried out by using FirstChoice[®] RLM-RACE kit, according to the manufacturer's instruction (Ambion, Austin, TX). The primer sequences used as outer and inner gene

specific primers were ATGTCGTTTCGGTCTCCTATGC and TCTGTGGAACGTCAGTGAGAGC, respectively.

2.3. RT-PCR

RT-PCR analyses were carried out by using the PCR primers specific for $Fc\alpha/\mu R$ (CF: CTCCTTTCAGGTACAAATGCA, CR: TCTGTGGAACGTCAGTGAGAGC),

$Fc\alpha/\mu R$ -L (LF: AAGCCCAGTGAACAAAAGGTCC, CR:

TCTGTGGAACGTCAGTGAGAGC), $Fc\alpha/\mu R$ -K (KF:

TGAAAACCTTGCCAGGACACGC, CR: TCTGTGGAACGTCAGTGAGAGC).

PCR primers specific for *Podocalyxin* (F: ACTACATTGCCCGTCTCCAC, R:

AAATCCTCAGCTGGCTTGAA) and *AQP1* (F:

CCTCCAGGCACAGTCTTCTC, R: CAGTGGCCTCCTGACTCTTC) were

also used.

2.4. Flowcytometry analyses

To examine the reactivity of each anti- $Fc\alpha/\mu R$ mAb against the $Fc\alpha/\mu R$

isoforms, 1×10^6 BW5147 transfectants expressing the Fc α/μ R isoforms were incubated with 0.05 μ g of biotin-conjugated anti-Fc α/μ R mAb, followed with FITC-conjugated streptavidin. To examine the ligand binding ability of each Fc α/μ R isoforms, 1×10^6 BW5147 transfectants expressing Fc α/μ R isoforms were incubated with 0.5 μ g of FITC-conjugated mouse IgM or IgA. To examine the blocking ability of anti-Fc α/μ R mAbs for ligand binding to the Fc α/μ R isoforms, 1×10^6 BW5147 transfectants expressing Fc α/μ R isoforms were incubated with 0.5 μ g of purified anti-Fc α/μ R mAb (TX57 or TX61) or control mouse IgG1 for 30 min at 4⁰C, followed with FITC-conjugated mouse IgM or IgA as above. To examine the Fc α/μ R expression on tubular cells, isolated tubular cells were stained with biotinylated-TX57 followed with APC-conjugated streptavidin and FITC-labeled anti-CD13 antibody. The cells were then analyzed by FACS Calibur^R (BD, San Diego, CA).

2.5. Immunohistochemistry

Frozen tissue of the kidney from wild type or $Fc\alpha/\mu R$ -deficient mice embedded in Tissue-Tek OCT compound (SAKURA Finetechnical, Tokyo, Japan) were cut into 5 μm sections by a cryostat. The sections were stained with biotin-conjugated anti- $Fc\alpha/\mu R$ mAb (TX7), followed by FITC-conjugated streptavidin (BD Pharmingen, CA) and analyzed under immunofluorescent microscopy.

2.6. Isolation of tubular cells from the kidney

The tubular cells were isolated as described previously (Baer et al., 1997). Briefly, the kidney tissue was fragmented to small pieces and incubated with RPMI1640 containing collagenase type I (1 mg/ml; GIBCO, Carlsbad, CA) and DNase (250 U/ml; Sigma-Aldrich, St. Louis, MO) for 45 minutes with gentle stirring. The digested tissue fragments were strained through a nylon mesh, suspended in PBS and overlaid on ice-cooled Percoll gradient 45% - 90%. After centrifugation at 2380 g for 30 minutes at 4^oC, cells at the lower interface were harvested. For flowcytometry analysis, CD13 was used for a marker of proximal tubular cells (Baer et al., 1997; Lacave et al., 1993). The

contamination of CD45⁺ blood cells were less than 1 % as determined by flow cytometry analysis.

2.7. Laser Micro Dissection (LMD)

Frozen tissue of the kidney embedded in Tissue-Tek OCT compound (SAKURA Finetechnical, Tokyo, Japan) were cut into 8 μ m sections by a cryostat. The sections were stained with toluidine blue (Muto Pure Chemicals, Tokyo, Japan), and glomerular and tubular regions were collected with Application Solutions Laser Microdissection System (Leica Microsystems, Wetzlar, Germany), according to the manufacturer's protocol.

3. Results and discussion

3.1. Identification of the *Fc α / μ R* isoform specifically expressed in the kidney

We previously demonstrated that *Fc α / μ R* was expressed in both lymphoid and non-lymphoid organs including lung, testis and kidney (Sakamoto et al., 2001). These results were reproducible in RT-PCR in mice on both C57BL/6J and BALB/c backgrounds when the primers specific to cDNAs derived from the exons 3 and 4 (designated CF and CR, respectively) were used (**Figs. 1A, B**). However, RT-PCR using the forward primer specific to cDNA derived from the exon 1 (designated LF) in combination with the reverse primer CR did not amplify the *Fc α / μ R* cDNA from the transcripts derived from the kidney (**Figs. 1A, B**). These results suggested that the kidney expressed only the isoform of the *Fc α / μ R* (*Fc α / μ R-K*), which did not conserve the N-terminal end that existed in *Fc α / μ R* expressed in the lymphoid organs (*Fc α / μ R-L*).

To analyze the molecular characteristic of *Fc α / μ R-K*, we carried out 5' RACE by using the two anti-sense primers specific to Exon 4 (designated

RACE-R (Fig.1A)), and determined the mouse cDNA sequence of $Fc\alpha/\mu R-K$ on the C57BL/6J background (**Fig. 1C**). By comparing the $Fc\alpha/\mu R-K$ cDNA sequence to the mouse genome resources data base of NCBI, the exon structure was predicted as shown in Fig. 1A, indicating that $Fc\alpha/\mu R-K$ seemed to use different exons 1 and 2 from those of $Fc\alpha/\mu R-L$ (**Fig. 1A**). The amino acid alignment showed that $Fc\alpha/\mu R-K$ harbored a different leader sequence from that of $Fc\alpha/\mu R-L$ and the putative mature protein contains the 25 amino acids stretch in the N-terminal end (**Fig. 1C**). RT-PCR analysis demonstrated that $Fc\alpha/\mu R-K$ was expressed only in kidney, but not in other tissues (**Fig. 1B**), from mice on the C57BL/6J background. Vice versa, $Fc\alpha/\mu R-L$ was expressed in all the organs expressing $Fc\alpha/\mu R$ except the kidney and heart (**Fig. 1B**). Of note, $Fc\alpha/\mu R-K$ was not detected in the kidney of mice on the BALB/c background, suggesting that BALB/c mice may express a different isoform in the kidney. These results indicated that $Fc\alpha/\mu R-K$ was specifically expressed in the kidney of mice on the C57BL/6J background.

3.2. *Functional characterization of the kidney-specific Fc α / μ R isoform*

To analyze the functional characteristics of Fc α / μ R-K, we established BW5147 transfectants stably expressing Fc α / μ R-L or Fc α / μ R-K. We previously generated twelve monoclonal antibodies against Fc α / μ R-L, which were divided into the five groups based on their recognition sites (Cho et al., 2006). Each monoclonal antibody from the five groups efficiently stained the transfectant expressing Fc α / μ R-K as well as Fc α / μ R-L (**Fig. 2 and data not shown**), indicating that each epitope that was recognized by the five groups of anti-Fc α / μ R-L was conserved in Fc α / μ R-K.

We next examined whether Fc α / μ R-K were able to bind to IgA and IgM. FITC-conjugated mouse IgA or IgM were incubated with the transfectants expressing Fc α / μ R-L or Fc α / μ R-K, and analyzed by flowcytometry. The transfectant expressing Fc α / μ R-K was stained with both IgA and IgM, and the binding of the IgA and IgM were comparable to those of the transfectant expressing Fc α / μ R-L (**Fig. 2A**). We previously reported that pre-incubation of transfectant expressing Fc α / μ R-L isoform with the Fc α / μ R-specific antibodies

TX57 and TX61 totally and partially blocked the ligand bindings, respectively (Cho et al., 2006). As shown in **Fig. 2B**, pre-incubation of transfectant expressing the $Fc\alpha/\mu R$ -K isoform with TX57 or TX61 blocked the IgA or IgM binding, as observed in the transfectant expressing $Fc\alpha/\mu R$ -L. These results suggest that $Fc\alpha/\mu R$ -L and $Fc\alpha/\mu R$ -K bind their ligands at the same site in the extracellular portion.

3.3. *Fc $\alpha/\mu R$ -K expression in the kidney*

To evaluate the localization of $Fc\alpha/\mu R$ -K in the kidney, immunohistochemical analyses of the kidney were performed. The signal of $Fc\alpha/\mu R$ protein was preferentially detected in the tubular epithelial cells. In contrast, it was not detected in the glomerular regions (**Fig. 3A**). We also analyzed $Fc\alpha/\mu R$ -deficient mice (Honda et al, submitted), as a negative control, which did not show any signal in tubular as well as glomerular regions (**Fig. 3A**). To confirm the expression of $Fc\alpha/\mu R$ in the tubuli, the tubular cells were isolated from the kidney and stained with a mAb against CD13, which was a marker of

proximal tubular cells (Baer et al., 1997; Lacave et al., 1993). The flow cytometry analyses demonstrated that $Fc\alpha/\mu R$ was detected on $CD13^+$, but not $CD13^-$, cells at a low level in wild type mice, as compared with negative controls using $Fc\alpha/\mu R$ -deficient mice (**Fig. 3B**). Taken together, these results indicated that $Fc\alpha/\mu R$ -K was specifically expressed on the tubular epithelial cells.

Because tubular epithelial cells are sticky and often nonspecifically bind antibodies, we further examined the specificity of $Fc\alpha/\mu R$ -K expression in the tubular epithelial cells. We separately excised the glomerular and tubular regions by the laser microdissection (LMD) system and analyzed them for expression of $Fc\alpha/\mu R$ -K by RT-PCR. As shown in **Fig. 4**, the tubular regions expressed the glomeruli-specific *podocalyxin*, but not tubule-specific *AQP 1* (Osafune et al., 2006). Reciprocally, the glomerular regions expressed *podocalyxin*, but not *AQP 1*, indicating that the separation of the glomerular regions from the tubular regions was successful. $Fc\alpha/\mu R$ -K was expressed only in the tubular, but not glomerular, regions. Taken together, these results indicated that $Fc\alpha/\mu R$ -K was specifically expressed on the tubular epithelial

cells.

A previous report showed that human mesangial cells expressed $Fc\alpha/\mu R$, which was upregulated by inflammatory cytokine stimuli (McDonald et al., 2002), suggesting that $Fc\alpha/\mu R$ may be the receptor responsible for mesangial IgA deposition in IgA nephropathy. On the other hand, Monteiro's group proposed a model whereby two types of IgA receptors that were soluble $Fc\alpha R$ (CD89) and transferrin receptor (CD71), rather than $Fc\alpha/\mu R$, participated in sequential steps to promote the development of IgA nephropathy (Moura et al., 2008). Although we have not determined the expression profile of $Fc\alpha/\mu R$ in human kidney, the present study supports that $Fc\alpha/\mu R$ may not be involved in IgA nephropathy. Rather, the expression of $Fc\alpha/\mu R$ in the tubular epithelial cells suggested that $Fc\alpha/\mu R$ might play an important role in immunity in the renal tubular regions.

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Figure legends

Fig. 1. Identification of kidney-specific Fc α / μ R isoform

(A) Alignment of the exon structures of the Fc α / μ R derived from lymphoid organs (Fc α / μ R-L) and kidney-specific Fc α / μ R isoform (Fc α / μ R-K). The arrowed lines indicate the primers used for RT-PCR analyses performed in B. (B) RT-PCR analyses for the transcripts of Fc α / μ R in the kidney and other organs in C57BL/6 and BALB/c mice. Total cDNA were obtained from each organ indicated, and RT-PCR analysis was carried out by using the primers specific for each Fc α / μ R isoform as shown in (A). (C) Alignment of predicted amino acid sequences of the 5' end of Fc α / μ R-K and Fc α / μ R-L. The putative leader sequences are underlined. The cDNA sequence data of Fc α / μ R-L and Fc α / μ R-K are available from EMBL/GenBank/DDBJ under accession number AB048834 and FJ011703, respectively.

Fig. 2. Binding of kidney-specific Fc α / μ R isoform with IgA and IgM

(A) BW5147 transfectants expressing Fc α / μ R isoforms indicated were stained

with biotinylated anti-Fc α / μ R mAb (TX61), followed with FITC-conjugated streptavidine, or with FITC-conjugated mouse IgA or IgM. Cells were then analyzed by flowcytometry. Shaded histograms indicate autofluorescence of the transfectants. (B) BW5147 transfectants expressing Fc α / μ R isoforms were incubated with purified anti-Fc α / μ R mAb (TX57 or TX61) or control mouse IgG1, followed with FITC-conjugated mouse IgM or IgA. IgA or IgM binding to the Fc α / μ R-transfectants in the presence (bold line) or absence (light shaded histograms) of each antibody were shown. Data are representative from three independent experiments.

Fig. 3. Immunohistochemistry of the kidney

(A) The frozen sections of the kidney from wild type or Fc α / μ R-deficient mice were stained with biotin-conjugated anti-Fc α / μ R mAb (TX7) or isotype-matched control IgG, followed by FITC-conjugated streptavidin and analyzed under immunofluorescent microscopy. The asterisks indicate the glomerular regions.

(B) The tubular cells were isolated from the kidney of wild type (bold line) or

$Fc\alpha/\mu R$ -deficient (shaded line) mice and stained with biotinylated-TX57, followed with APC-conjugated streptavidin and FITC-labeled anti-CD13 antibody. $Fc\alpha/\mu R$ expression on CD13⁺ and CD13⁻-gated cells were shown. Data are representative from three independent experiments.

Fig. 4. Expression of $Fc\alpha/\mu R$ isoform in the kidney

(A) Frozen tissue sections from the kidney were stained with toluidine blue, and glomerular (upper column) and tubular (lower column) regions were separately excised by laser microdissection system (LMD), as indicated. The asterisks indicate the glomerular regions. (B) Total cDNA was extracted from each excised tissue by LMD and subjected to RT-PCR analyses by using primers specific for each gene indicated.