Monocyte chemoattractant protein (MCP)-1 production via functionally reconstituted Fcα receptor (CD89) on glomerular mesangial cells

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Abstract. Background: Fc alpha receptor (FcαR; CD89) is the receptor for Fc portion of IgA in various cells, and displays various immunological responses on binding. It is important to analyze the mesangial functions via FcαR in the pathogenesis of IgA nephropathy. However, it is still controversial whether FcαR is expressed on mesangial cells. To assess biological functions of FcαR on the mesangial cells, we established mesangial transfectants that expressed FcαR with or without FcRγ chain that is a common signaling molecule of FcRs. The production of monocyte chemoattractant protein-1 (MCP-1) by mesangial cells is known to contribute to cellular infiltration into glomeruli and subsequent glomerular injuries.

Methods: Murine mesangial cell lines (SV40 MES 13) were transfected with cDNA of the human FcαR. Furthermore, we co-transfected some of the FcαR transfectants with cDNA of human FcRγ chain. The tyrosine phosphorylation of the intra-mesangial proteins after FcαR cross-linking was examined by immunoprecipitation. MCP-1 production from each transfectant stimulated with heat aggregated IgA was determined by sandwich ELISA.

Results: Two kinds of mesangial transfectants stably expressed human FcαR with or without FcRγ chain (FcαR+, FcαR+/γ). Phosphorylation of FcRγ chain and syk kinase was detected in FcαR+ and FcαR+/γ cells, but not in untransfected cells. Aggregated IgA induced significantly higher MCP-1 production in FcαR+/γ+ than those in FcαR+ or untransfected control.

Conclusion: Present study demonstrated that FcαR and FcRγ chain could be reconstituted in mesangial cells and mediated MCP-1 production by aggregated IgA in a dose-dependent manner. Current data would argue that FcαR can be activated in mesangial cells through their own machinery, although underlying mechanisms for FcαR induction in mesangial cells remain unclear.

Key words: CD89, MCP-1, Mesangial cells, IgA nephropathy

Introduction

IgA nephropathy is characterized by the depositions of aggregated IgA or IgA immune-complexes in the glomerular mesangial areas with mesangial cell proliferation and matrix expansion [1]. It is not clear if only IgA deposits in glomeruli are responsible for the glomerular inflammatory changes characteristic of the advanced stage of IgA nephropathy. The precise mediators for signaling lymphocytes and monocytes to migrate and colonize the kidney are not well known. Recent findings that monocyte chemoattractant protein-1 (MCP-1), a chemoattractant cytokine with a high degree of specificity for lymphocytes and monocytes, is overexpressed in glomeruli from rats with immune-complex glomerulonephritis prompted us to explore the possibility that MCP-1 might be implicated in the renal inflammatory response [2–5].

Several studies have clarified that Fc receptors (FcRs) lead to cell activation by multivalent molecules with immunoglobulins and play a key role in immunoglobulin-mediated inflammation such as allergic diseases. Moreover, recent studies revealed that FcR may play an important role in experimental glomerulonephritis using FcRγ chain knockout mice [6]. On the other hand, FcR for IgA (FcαR; CD89) might induce various immunological responses such as antibody dependent cellular cytotoxicity (ADCC) [7], and secretion of cytokines and chemokines [8, 9]. FcαR is glycosylated membrane protein of 50–75 kDa, which is expressed mainly on monocytes, neutrophils and eosinophils [10]. Previous studies reported that the FcRγ chain is essential for signal transduction via FcαR with IgA in transfected murine B cell and human monocyteic cell lines [11, 12]. We verified the physical association of FcαR and the FcRγ chain on human glomerular mesangial cells and hypothesized their function—
al expressions [13]. A Spanish group reported that FcαR on human cultured mesangial cells might induce cell-activation [14–16], although it is still uncertain whether the FcαR activation through FeRγ chain on mesangial cells is actually induced. The purpose of the present study is to investigate whether FcαR (with the FeRγ chain) introduced on mesangial cells can be activated and induce the phosphorylation of the FeRγ chain or syk and produces the monocyte chemotactrant-protein-I (MCP-1) through mesangial machinery.

Materials and methods

Cells and cell culture

The murine glomerular mesangial cell line, SV40MES13, was maintained with DMEM/HAM’sF12 supplemented with 5% heat-inactivated FCS. FcαR with/without FeRγ chain transfected cells were maintained in the same medium supplemented with genetecin; G418 (200 µg/ml) and hygromycin B (200 µg/ml) (Wako Chemical Co., Ltd., Tokyo, Japan). Murine mast cell line (PT 18) and human monocytoid cell line (U937, ATCC, Rockville, MD, USA) were cultured in RPMI 1640 with 10% FCS. For some experiments, U937 cells were treated with 10−<sup>7</sup> M phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical Co., St. Louis, Mo, USA) and with 400 U/ml of human recombinant interferon-γ (IFN-γ) (Shionogi, Osaka, Japan) for 16 h [12].

Transfection with expression vectors

We generated pMkitNeo vectors containing the human FcαR chain cDNA (pMkitNeo/FcαRα) and pMikhgygro vectors containing human FeRγ chain cDNA (pMIKhygro/FeRγ). Both vectors without these cDNAs (pMkitNeo, pMIKhygro) were used as controls. We introduced these genes into SV40MES13 cells by calcium phosphate precipitation methods. After selection in the medium with G418 or hygromycin B, several clones were picked up using a cloning ring (Iwaki Glass Co. Ltd., Tokyo, Japan). We finally established three kinds of stable-transfectants as follows: both control vectors (FcαRα/γ, pMkitNeo/FcαRα and pMIKhygro (FcαRα), or pMkitNeo/FcαRα and pMIKhygro/FeRγ chain (FcαRα/γ)).

Expression of FcαRα and FeRγ chains in transfectants

Expression of human FcαR and FeRγ chains in transfectants were confirmed by Northern blot analysis. Total RNA (10 µg) from each transfectant was electrophoresed on 1% agarose gels containing 10% formaldehyde and transferred onto nitrocellulose membranes (Gene Screen, NEN Life Science Products, Boston, MA, USA). Probes for FcαR and FeRγ chain were labeled with [γ<sup>32</sup>P]dCTP (Amersham Pharmacia). The membranes were hybridized for 16 h at 42°C in labeled probe, 40% formamide solution, 0.5% SDS, 6X SSPE, 5X Denhardt’s solution and 0.25 mg/ml of salmon sperm DNA (Sigma). The hybridized membrane was washed three times for 30 min in 2XSSC/0.1% SDS at room temperature and 0.1XSSC/0.1% SDS at 55°C and 0.1XSSC/0.1% SDS at 65°C and then autoradiographed using Fujix Bass 2000 system (Fuji photofilm co.ltd. Tokyo, Japan).

Physical association of FcαRα and FeRγ chains in transfectants

These transfectants were lysed in 1% digitonin (1% digitonin, 150 mM NaCl, 50 mM Tris-HCl, pH 7.5) containing the protease inhibitors (1 mM PMSF, 1 mM pepstatin, 1 mM aprotinin) for 40 min on ice. Insoluble cell lysates were discarded by centrifugation at 15,000 g for 10 min and then precleared with protein G-Sepharose 4B beads (Pharmacia P-L Biochemicals Inc., Milwaukee, WI, USA) at 4°C for 2 h under constant rotation. The precleared lysates were then subjected to immunoprecipitation with monoclonal anti-human FcαR antibody (AAR1) [12]. The immunoprecipitates were collected on protein G-Sepharose 4B beads, washed with digitonin buffer, and then extracted from the beads by boiling in SDS sample buffer with or without 2-mercaptoethanol (2ME). They were analyzed by SDS-PAGE (15% polyacrylamide gel) and electrophoretically transferred to the membrane (Pall Fluoro Trans. W Membrane, Nippon Genetics, Tokyo, Japan). Detection of the association was finally determined by anti-FcαR antibody and subsequent second antibody (HRP-conjugated anti-rabbit IgG antibody) in ECL reagent (Amersham Life Science, Buckinghamshire, UK). Molecular mass markers were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

Analyses of protein tyrosine phosphorylation

Transfectants were washed twice in PBS and resuspended at 10<sup>6</sup> cells/ml in RPMI 1640 without serum. After the incubation with 10 µg/ml biotinylated AAR1 for 30 min on ice, excess antibody was removed by cold PBS. Then, the cells were incubated at 37°C for 5 min with 20 µg/ml of strept-avidin for stimulation of FcαR. They were rapidly washed with PBS containing 10 mM NaF and 400 µM Na<sub>2</sub>VO<sub>4</sub>. The reaction was stopped by the addition of boiling SDS-PAGE sample buffer containing 100 mM NaF, 1 mM Na<sub>2</sub>VO<sub>4</sub>, and protease inhibitors. These lysates were heated at 95°C for 5 min and centrifuged to remove the insoluble proteins. The samples were separated by SDS-PAGE and electrophoretically transferred to the filter. Final detection was achieved by incubation with anti-phosphotyrosine antibody (4G10) and subsequent HRP-conjugated anti-mouse IgG antibody in ECL reagent.

In the immunoprecipitation for detection of phosphorylated molecules, the stimulated cells were lysed on ice in lysis buffer (1% NP-40, 150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, protease inhibitors, 1 mM Na<sub>2</sub>VO<sub>4</sub> and 100 mM NaF) for 40 min. Insoluble proteins were discarded by centrifugation at 15,000 g for 10 min. The lysates were precleared with 20 µl of protein A-Sepharose 4B beads (Pharmacia P-L Biochemicals Inc., Milwaukee, WI, USA) at 4°C for 2 h under constant rotation. The precleared lysates were then immunoprecipitated at 4°C for 2 h with anti-FcαR chain antibody or anti-syk antibody coupled protein A-Sepharose 4B beads. The immunoprecipitated beads were washed with NP-40 buffer containing 1 mM Na<sub>2</sub>VO<sub>4</sub>. These immunoprecipitates were extracted from the beads by boiling in SDS sample buffer with or without 2-ME, subjected to SDS-PAGE after boiling and electrophoretically transferred to the filters. These filters were then incubated with monoclonal anti-phosphotyrosine antibody (4G10:1 µg/ml). After washing with 0.05% Tween 20, HRP conjugated anti-mouse IgG antibody was used as secondary antibody. Finally, the filters were washed and then developed in an ECL PLUS (Amersham) chemiluminescence detection system.

Measurement of MCP-1 production by each transfectant

Heat aggregated IgA was generated by the incubation of human myeloma IgA (Cappel, ICM Pharmaceuticals, Inc., Aurora, OH, USA) at 63°C for 150 min. After centrifugation, insoluble aggregates were discarded [16]. Transfectants were cultured in RPMI 1640 with 5% FCS for 24 h. After washing with the medium, each transfectant was incubated with the heat-aggregated IgA at different concentrations (0, 10, 25, 50, 100 and 200 µg/ml) for 12 h. Concentrations of MCP-1 in the culture supernatants from each stimulated transfectant were determined by sandwich ELISA. For compensation, each concentration of MCP-1 was divided by each protein concentration of the cell lysates.