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TECHNICAL NOTE

Real-time monitoring of mesangial cell–macrophage cross-talk using SEAP in vitro and ex vivo

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Real-time monitoring of mesangial cell–macrophage cross-talk using SEAP in vitro and ex vivo.

Background. Macrophage–mesangial cell interaction plays a crucial role in the pathogenesis of glomerulonephritis. We established a novel system for continuous, real-time monitoring of cross-talk between macrophages and mesangial cells in vitro and ex vivo.

Methods. Rat mesangial cells were genetically engineered to produce secreted alkaline phosphatase (SEAP) under the control of the nuclear factor-κB (NF-κB) enhancer elements. The established sensor cells were exposed to macrophages or macrophage-derived factors, and the level of SEAP production was evaluated.

Results. In vitro, the established cells expressed and secreted SEAP when exposed to activated macrophages or to cytokines produced by macrophages. The kinetics of SEAP activity in culture media was closely correlated with the expression level of SEAP mRNA. The sensor cells also secreted SEAP in response to media conditioned by macrophage-accumulating, inflamed rat glomeruli. When the sensor cells were transferred adoptively into rat glomeruli subjected to acute anti-Thy 1 glomerulonephritis, the isolated glomeruli containing sensor cells secreted SEAP rapidly and progressively.

Conclusion. These data suggested that the established system provides simple and useful tools for monitoring of cross-talk between macrophages and mesangial cells in vitro and ex vivo. This approach would be useful for investigation of molecular mechanisms involved in mesangial cell–macrophage interaction and also for screening of therapeutic agents that efficiently interfere with the link between infiltrating leukocytes and resident glomerular cells.

Key words: biosensor, mesangial cell, macrophage, nuclear factor-κB, secreted alkaline phosphatase.

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Monocytes/macrophages play a crucial role in the generation of various glomerulonephritis [1]. Under pathologic situations, activated macrophages elaborate a variety of inflammatory mediators and stimulate resident cells, leading to initiation and progression of glomerular injury [2]. In particular, activation of mesangial cells is essential for mesangial proliferation and matrix expansion, the typical pathologic features of proliferative glomerulonephritis. Using a technique for in vivo transfer of genetically engineered macrophages, we previously demonstrated that, within glomeruli, activated macrophages induce expression of activation markers in resident glomerular cells and that this effect was dependent on activation of nuclear factor-κB (NF-κB) in macrophages [3–6]. Using techniques for macrophage depletion and adoptive macrophage transfer, a recent report also provided direct evidence showing that activated macrophages trigger mesangial cell proliferation and proteinuria. [7]. From this viewpoint, monitoring of macrophage–mesangial cell cross-talk is important for evaluation of activity of glomerulonephritis as well as for understanding of mechanisms underlying glomerular injury. In this report, we aimed at creating a novel system that allows for continuous, real-time monitoring of cross-talk between macrophages and mesangial cells in vitro and ex vivo. For this purpose, a sensor mesangial cell line was created by using the κB enhancer element as a molecular sensor and secreted alkaline phosphatase (SEAP) as a reporter molecule. Because activated macrophages release various molecules that trigger activation of NF-κB in resident cells, the κB site can be used as a sensor sequence for macrophage–mesangial cell interaction.

SEAP, originally reported by Berger et al [8], has been used for monitoring activity of known or putative enhancer/promoter elements in vitro. Normally, alkaline phosphatase is not secreted, but the recombinant SEAP derived from placental alkaline phosphatase is efficiently

secreted from transfected cells. In SEAP-transfected cells, the level of SEAP activity detected in culture media is directly proportional to changes in intracellular SEAP mRNA [8, 9]. This property allows SEAP to serve as a quantitative reporter for gene expression. As a reporter gene, SEAP has several important advantages over other reporter molecules including chloramphenicol acetyltransferase, luciferase, green fluorescent proteins, and β-galactosidase. Because preparation of cell lysates is not required, it is possible to monitor the activity of certain promoters/enhancers continuously using the identical cell cultures. Assaying SEAP activity using culture medium is faster, easier, and less expensive than assaying other reporter enzymes. Using chemiluminescent assays, detection of SEAP activity is extremely sensitive [10]. Unlike endogenous alkaline phosphatases, SEAP is heat-stable and resistant to its inhibitor L-homoarginine [9]. The influence of endogenous alkaline phosphatases can be eliminated completely by preheating the sample at 65°C and assaying in the presence of L-homoarginine without affecting the SEAP activity.

To establish a system for continuous monitoring of macrophage–mesangial cell interaction, we stably introduced the SEAP gene into mesangial cells under the control of the κB enhancer elements. Our data demonstrated that the established systems are useful for in vitro monitoring of macrophage–mesangial cell cross-talk and also for ex vivo monitoring of their interaction within nephritic glomeruli where macrophages play crucial, pathogenic roles.

METHODS

Reagents

Human recombinant interleukin-1β (IL-1β) and human recombinant tumor necrosis factor-α (TNF-α) were generous gifts of Otsuka Pharmaceutical Co. Ltd (Tokushima, Japan) and Dr. Katsuo Noguchi (Teikyo University School of Medicine, Tokyo, Japan), respectively. 12-*o*-tetradecanoylphorbol-13-acetate (TPA) was purchased from Sigma-Aldrich Japan (Tokyo, Japan), human platelet-derived growth factor (PDGF)-BB was from Perro Tech, Inc. (Rocky Hill, NJ, USA), forskolin and lipopolysaccharide (LPS) (*Escherichia coli* 0111) (B4) were from Sigma Chemical Co. (St. Louis, MO, USA), and MG132 was from Peptide Institute (Osaka, Japan).

Cells

Clonal mesangial cells (SM43) were established from isolated renal glomeruli of a male Sprague-Dawley rat and identified as being of the mesangial cell phenotype as described before [11]. The normal alveolar macrophage cell line NR8383 [12] derived from a Sprague-Dawley rat was a gift from Dr. Seishiro Hirano (National Institute for Environmental Studies, Tsukuba, Japan). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/F-12 (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 100 U/mL penicillin G, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, and 5% to 10% fetal bovine serum (FBS). Medium containing 1% FBS was generally used for studies.

Stable transfection

Using a calcium-phosphate coprecipitation method [13], mesangial cells (passage 10 to 15) (5×10^5 cells) were transfected with pNF-κB-SEAP (5 μg) (BD Biosciences, Palo Alto, CA, USA) together with pcDNA3.1 (1 μg) (Invitrogen, Carlsbad, CA, USA) that encodes neomycin phosphotransferase gene. pNF-κB-SEAP encodes SEAP under the control of 4 copies of the κB enhancer element. Stable transfectants were selected by 330 μg/mL G418, and a clone with the highest SEAP inducibility was selected and used for studies. We designated this sensor clone as SM/NF-κB-SEAP5. The mesangial cell phenotype of this clone was confirmed by positive staining for α-smooth muscle actin (α-SMA) and Thy 1.1 using an anti-α-SMA monoclonal antibody (1:300 dilution) Sigma Chemical Co.) and an anti-Thy 1.1 monoclonal antibody 1-22-3 (1:100 dilution) [14]. Using the same method, SM/CRE-SEAP15 cells were established by transfection of mesangial cells with pCRE-SEAP (BD Biosciences) together with pcDNA3.1. pCRE-SEAP encodes SEAP under the control of three copies of the cyclic adenosine monophosphate (cAMP) response element (CRE). Mock-transfected mesangial cells expressing *neo* alone were established by transfection with pcDNA3.1. Stably transfected cells between passage 20 and 35 were used for experiments.

Pharmacologic treatments

Sensor mesangial cells were stimulated by 1 pg/mL to 20 ng/mL IL-1β for 0.5 to 24 hours, and the cells and culture media were subjected to Northern blot analysis and SEAP assay, respectively. To examine selectivity of the sensor cell response, cells were exposed to stimulators of the NF-κB enhancer element, IL-1β and TNF-α (20 ng/mL and 250 U/mL, respectively); a stimulator of the TPA response element (TRE), TPA (50 ng/mL); a stimulator of CRE, forskolin (10 μmol/L); and a stimulator of the serum response element (SRE), PDGF-BB (20 ng/mL).

Northern blot analysis

Total RNA was extracted by a single-step method [15], and Northern blot analysis was performed as described