Gene Expression Profile in Experimental Mesangial Proliferative Glomerulonephritis

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Abstract. We analyzed gene expression in rat anti-Thy1 antibody-induced glomerulonephritis by using the cDNA microarray method. Ninety-seven genes that differed by more than 1.5-fold intensity in comparison with the controls were selected. Cluster analysis showed that the expression of genes associated with inflammation reached maximum levels at 24 h, while genes involved in the development of fibrosis increased at 7 days after injection. Microarray analysis of animal disease models may be a powerful approach for understanding the gene expression programs that underlie these disorders.

Keywords: cDNA microarray, nephritis, gene expression

Accumulating evidence indicates that increased expression of several cytokines and growth factors contributes to the progression of glomerulonephritis (GN) (1, 2). Although immunoglobulin A (IgA) nephropathy is the most common primary renal glomerular disease, there are not any effective drugs for IgA nephropathy as yet. Anti-Thy1 antibody-induced GN is one of the experimental models of IgA nephropathy. The pathohistology of anti-Thy1 antibody-induced GN has been well characterized. However, previous studies of gene expression have generally been limited to analysis of one or a few genes. Therefore, we have been provided little information about the pattern of gene expression involved in this response. In the present study, we analyzed gene expression in the kidneys of anti-Thy1 antibody-induced rats by using cDNA microarrays with a set of 3907 cDNA clones.

Female Wistar rats (Clea Japan, Tokyo), weighing 120 – 140 g, were used. All procedures were in accordance with institutional guidelines for animal research. Experimental mesangial proliferative GN was prepared by an injection of anti-Thy1 antibody (clone OX-7; PharMingen, San Diego, CA, USA), 1 mg/kg of body weight, to rats via the tail vein. Rats were sacrificed at 3, 6, 24, 72, and 168 h after anti-Thy1 antibody injection (n = 7 or 8 at each time point). Age- and weight-matched Wistar rats were injected with an equal volume of saline. After killing, bilateral kidneys were immediately removed. Then, glomeruli were isolated from bilateral kidneys by the sieving method, as previously reported (3).

Histological examination was performed on 3-μm sections of paraffin-embedded tissues stained with periodic acid-Schiff (PAS). At 7 days after anti-Thy1 antibody injection, the mesangial cells had markedly proliferated and Bowman’s space had narrowed by expansion of the mesangial matrix (Fig. 1A).

Total RNA of glomeruli was extracted by using Isogen (Nippon Gene, Toyama). cDNA clones were isolated from a rat cDNA library. To exclude redundancy from the kidney cDNA library, it was normalized by the subtraction method using drivers as described previously (4, 5). Sequencing of the clones was performed using the MegaBACE 1000 DNA Sequencing system (Amersham Biosciences Corp., Piscataway, NJ, USA). cDNA fragments amplified by the polymerase
chain reaction (PCR) were spotted onto glass slides using a robotic mechanism. A total of 3907 clones were printed on our kidney chip. We hybridized labeled target DNAs on the cDNA microarrays. Hybridization images were scanned by GenePix 4000A (Axon Instruments, Union City, CA, USA) and the signal intensities were quantified with GenePix Pro 3.0. At least two hybridizations were performed at each condition. Data analysis was performed as previously described (5). Signal intensities were converted by taking logarithms of base two. The given residuals explain logarithmic gene expression ratios. We selected 97 genes that differed by more than 0.58 or less than −0.58, i.e., 1.5-fold difference in at least one pairwise comparison. The number of differentially regulated clones at each time point is shown in Fig. 1B. This result shows that most of the selected genes were upregulated at 24 or 72 h.

We classified the selected clones into six clusters by using GeneMaths software (Fig. 1C). Cluster 1 included genes of which expression was downregulated at the late phase (72 or 168 h) after anti-Thy1 antibody injection. Clones in cluster 1 were globin protein-encoded genes and signal transduction proteins, such as extracellular signal-regulated kinase 1 and MEK.
kinase 3. Cluster 2 included genes whose expressions were downregulated at the early phase after anti-Thy1 antibody injection and then returned to the basal level. Thy1 antigen was contained within cluster 2. Cluster 3 or 4 involved genes whose expressions were upregulated at 168 or 72 h, respectively, after anti-Thy1 antibody injection. Genes in cluster 3 were composed of extracellular matrix-related genes, such as collagen and fibronectin. Cluster 5 involved genes whose expressions were upregulated mainly 24 h after injection. Cluster 5 included inflammation-related genes, such as osteopontin, complement C3 (6) and IgE binding protein, and chemokine CX3C (7). Cluster 6 involved genes whose expressions were upregulated 6 h after injection and then returned to the basal level.

To confirm the microarray analysis, we performed reverse transcription-PCR (RT-PCR) analysis of several differentially regulated genes by using glomerular RNA derived from saline- or anti-Thy1 antibody-injected rats (Fig. 2). These RT-PCR results confirmed the differential expression pattern of several genes identified by the cDNA chip system.

Using these approaches, we identified several genes that were more highly expressed in GN. At the early phase after anti-Thy1 antibody injection, gene expression of migratory inhibitory factor-related protein 14 (MRP14) was remarkably increased, compared with the control. Calcium-binding proteins such as MRP8 and MRP14 are markers of monocytes/macrophages and neutrophils in acute and chronic inflammation (8, 9). A complex of MRP 8/14 rapidly formed via calcium influx and protein kinase C (PKC) activation. PKC activated mitogen-activated protein (MAP) kinases. Taken together with our previous report that glomerular extracellular signal-regulated kinase (ERK) and c-Jun NH2-terminal kinase (JNK), belonging to MAP kinases,
are significantly activated in GN (3), the increase of MRP8/14 mRNA expression in anti-Thy1 GN might be mediated by activation of MAP kinases. Therefore, MRP14 seems to be a very early marker and an important regulator in anti-Thy1 GN. However, additional study is needed to elucidate the precise mechanism underlying the increase of these gene expressions via MAP kinases.

Chemokines are a family of proteins that chemoattract and active cells by interacting with specific receptors on the surface of their targets. They are grouped into four classes based on the position of key cysteine residues: C, CC, CXC, and CX3C (7). Stromal cell-derived factor 1 (SDF1), the ligand of the CXCR4 receptor, is a highly conserved gene whose protein is chemotactic for T lymphocytes, monocytes, and neutrophils (10). Our data showed that CX3C mRNA expression is increased at 24 h. Expression of CXC motif ligand 4 (CXCL4), whose receptor is CXCR4, also tended to be increased at 24 h, compared with the control. These results indicated that chemokines such as CX3C and CXCR4 are also targets for therapeutic intervention in GN. Thus, cDNA microarray analysis is a powerful approach for monitoring molecular events in various diseases.

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**References**