

## Dexamethasone Regulates AP-1 to Repress NF- $\kappa$ B and AP-1 Interdependent TNF- $\alpha$ Induced MCP-1 Production in Human Glomerular Endothelial Cells

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Glomerular endothelial cells may play a central role in the development of glomerulonephritis. For the induction of MCP-1, both transcription factors NF- $\kappa$ B and AP-1 were required in several cell types. However, in glomerular endothelial cells, little has been evaluated for intracellular signal transduction in MCP-1 production and the effect of glucocorticoid on those intracellular signaling process.

In the present study, we investigated whether the increase of MCP-1 in cultured human glomerular endothelial cells (HGEC) by TNF- $\alpha$  could be mediated by NF- $\kappa$ B and/or AP-1 by using decoy oligodeoxynucleotide, and whether these processes could be modified by glucocorticoid.

The levels of MCP-1 mRNA and MCP-1 were measured by Northern blot and sandwich ELISA, respectively. NF- $\kappa$ B and AP-1 were assessed by electrophoretic mobility shift assay (EMSA), and cytosolic I $\kappa$ B $\alpha$  and nuclear p65 protein were measured by Western blot.

As results, TNF- $\alpha$  (10ng/ml) increased the mRNA expression of MCP-1 in HGEC and the release of MCP-1 as compared to control (266 $\pm$ 24%, mean $\pm$ S.E., p<0.01, n=10, each n is duplicate), which were partially inhibited by dexamethasone (10nM) (190 $\pm$ 31% vs 266 $\pm$ 24%, mean $\pm$ S.E., p<0.05, n=10). TNF- $\alpha$  induced MCP-1 production appeared to be NF- $\kappa$ B and AP-1 interdependent based on the following results. (1) TNF- $\alpha$  increased NF- $\kappa$ B and AP-1 binding activity in 15-30 min after stimulation. (2) NF- $\kappa$ B decoy oligodeoxynucleotide (5'-AGTTGAGGGGACTTTCCAGGC-3'), and AP-1 decoy oligodeoxynucleotide (5'-CGCTTGATGACTCAGCCGAA-3'), partially suppressed TNF- $\alpha$  induced MCP-1 mRNA expression in Northern blot, whereas mismatched control did not suppress MCP-1 mRNA expression (n=3). The repeated EMSA showed that dexamethasone decreased TNF- $\alpha$  induced DNA binding activity of AP-1 (n=3) but not that of NF- $\kappa$ B (n=9). In addition, dexamethasone had no effect on cytosolic I $\kappa$ B $\alpha$  degradation, or p65 nuclear translocation (n=3).

These data demonstrated that TNF- $\alpha$  induced MCP-1 production was mediated by cooperative action of NF- $\kappa$ B and AP-1 in HGEC, and dexamethasone repressed TNF- $\alpha$  induced MCP-1 production via suppression of binding activity of AP-1 to DNA, not via NF- $\kappa$ B pathway in HGEC.