The Effect of Dexamethasone and TGF-1 on The Cytokine-induced production of MCP-1 and The Activity of NF-κB in Human Glomerular Endothelial Cells

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Background : Since glomerular endothelial cells are exposed directly to circulating factors and closely apposed to glomerular basement membrane and mesangial cells, it is likely that glomerular endothelial cells play a role in the pathogenesis of glomerulonephritis. Monocyte chemoattractant protein-1(MCP-1) is a chemotactic and activating peptide highly specific for monocyte, which can be produced in glomerular endothelial cells. Glomerular infiltration of monocytes/macrophages has been emphasized to cause glomerular injuries. It is therefore likely that MCP-1 may be an important factor on the pathogenesis of immune-mediated glomerulonephritis. It has been argued that glucocorticoids and TGF-1 can decrease the production of MCP-1 in many cells. The induction of MCP-1 is known to be regulated by NF-B at the transcriptional level. The activation of NF-B involves the targeted degradation of IB, and the translocation of NF-B to the nucleus. However, so far, little has been known about the effect of glucocorticoids and TGF-1 on the activity of NF-B and IB in human glomerular endothelial cells(HGECs). In the present study, we investigated whether glucocorticoids and TGF-1 could modify the cytokine-induced production of MCP-1 and activity of NF-B, which is known to be a major mediating transcription factor for MCP-1.

Methods : Human glomerular endothelial cells(HGECs) were isolated from a normal portion of nephrectomized tissues from patients with renal cell carcinoma. Endothelial cells were identified by immunohistochemical staining with rabbit antihuman factor VIII antibody, and the ability to take up fluorescent labeled (1,1-dioctadecyl-1-1-3,3,3,3,-tetramethyl-indocarbocyanine perchlorate)-acetylated low-density lipoprotein. The MCP-1 was quantified using sandwich ELISA and Northern blot analysis of MCP-1 mRNA. Then, dexamethasone and TGF-1 dependent activity of IB and NF-B was analyzed by Western blot and electrophoretic mobility shift assay (EMSA).

Results : TNF-(10ng/ml) increased the release of MCP-1 into culture supernatant as compared to control(26624%, meanS.E., $p<0.01$, n=10, each n is duplicate). Dexamethasone(10nM) partially inhibited the TNF--induced production of MCP-1(19031% vs 26624%, meanS.E., $p<0.05$, n=10). However, TGF-1(1ng/ml) did not decrease the TNF--induced production of MCP-1(25731% vs 26624%, meanS.E., $p>0.05$, n=10). Same results could be obtained in 0.1 and 10ng/ml of TGF-1(n=4). In northern blot analysis, Dexamethasone could partially inhibit

the induction of MCP-1 mRNA. But, TGF-1 did not decrease the induction of MCP-1 mRNA. In western blot analysis, after HGECs were treated with TNF-, cytoplasmic IB nearly completely disappeared within 15min of stimulation and reappeared in the cytoplasm after 30 min. In case of p65, after 15-30 min of TNF- exposure, p65 gradually appeared in the nuclear proteins, which was persistent until 2h. After pre-incubation for 12h with dexamethasone(10nM) and TGF-1(10ng/ml), HGECs were treated with TNF- (10ng/ml) as indicated time. This experiment showed that dexamethasone and TGF-1 had no influence to cytosolic degradation of IB, and nuclear translocation of p65 in TNF--stimulated HGECs. To determine the intracellular mechanism of the inhibitory effect of MCP-1 expression by dexamethasone, nuclear proteins were isolated and tested for NF-B binding activity by EMSA. TNF-increased the binding activity of nuclear protein to NF-B oligonucleotide from 5-15min up to 2h. However, after pretreatment of dexamethasone(10nM) and TGF-1(10ng/ml), TNF--induced binding activity of nuclear protein to NF-B was decreased by dexamethasone, but not by TGF-1.

Conclusions : From these experiments, we might confirm that in HGECs, dexamethasone has no influence on the degradation of IB and nuclear translocation of NF-B. However, direct interaction between the dexamethasone and NF-B is likely to be the underlying inhibitory mechanism of NF-B activity by dexamethasone in HGECs, which was expressed as an inhibitory production of MCP-1. We also could make a conclusion that TGF-1 had no effect to NF-B activity, therefore the production of MCP-1 in cultured HGECs, which would be a different result from the other studies using different cells.