MAPK phosphatase-1 represents a novel anti-inflammatory target of glucocorticoids in the human endothelium

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ABSTRACT **Glucocorticoids are well-established anti-inflammatory drugs thought to mainly act by inhibition of proinflammatory transcription factors like NF-κB. In recent years, however, transcription factor-independent mechanisms of glucocorticoid action have been proposed, namely the influence on MAPK pathways. Here we identify MAPK phosphatase-1 (MKP-1) as a pivotal mediator of the anti-inflammatory action of glucocorticoids in the human endothelium. We applied dexamethasone (Dex) to TNF-α-activated human endothelial cells and used the adhesion molecule E-selectin as inflammatory read-out parameter. Dex is known to reduce the expression of E-selectin, which is largely regulated by NF-κB. Here, we communicate that Dex at low concentrations (1–100 nM) markedly attenuates E-selectin expression without affecting NF-κB. Importantly, Dex is able to increase the expression of MKP-1, which causes an inactivation of TNF-α-induced p38 MAPK and mediates inhibition of E-selectin expression. In endothelial MKP-1$^{-/-}$ cells differentiated from MKP-1$^{-/-}$ embryonic stem cells and in MKP-1-silenced human endothelial cells, Dex did not inhibit TNF-α-evoked E-selectin expression. Thus, our findings introduce MKP-1 as a novel and crucial mediator of the anti-inflammatory action of glucocorticoids at low concentrations in the human endothelium and highlight MKP-1 as an important and promising anti-inflammatory drug target.—Fürst, R., Schroeder, T., Eilken, H. M., Bubik, M. F., Kiemer, A. K., Stefan Zahler, S., Vollmar, A. M. MAPK phosphatase-1 represents a novel anti-inflammatory target of glucocorticoids in the human endothelium *FASEB J.* 21, 74–80 (2007)**

Key Words: inflammation

GLUCOCORTICOIDS, SUCH AS CORTISOL, are endogenous agents produced by the adrenal gland. Glucocorticoids are essential for normal development and exert influence on various metabolic and immune defense processes. Synthetic glucocorticoids, like dexamethasone (Dex), are well established and widely used immunosuppressive and anti-inflammatory drugs. Glucocorticoids have been proven to exert beneficial effects in different autoimmune diseases and in a plethora of disorders with an inflammatory component, *e.g.*, chronic allergic diseases, asthma, rheumatoid arthritis, Crohn's disease, or chronic ulcerative colitis.

Glucocorticoids act by binding to their intracellular glucocorticoid receptor. Subsequent to its ligand-dependent activation, the receptor translocates to the nucleus and acts *1)* as a transcription factor upon binding to glucocorticoid response elements of distinct gene promotors or *2)* as a direct inhibitor of proinflammatory transcription factors like NF-κB, a protein-protein interaction known as transrepression (1). Metabolic effects of glucocorticoids, representing most of the adverse effects of glucocorticoid therapy, are mainly ascribed to the transcriptional activity of the glucocorticoid receptor, whereas the therapeutically favored anti-inflammatory actions are thought to be predominantly caused by the mechanism of transrepression (2). Recently, a new concept of "non-transcriptional" anti-inflammatory actions of glucocorticoids emerged: the activated glucocorticoid receptor initiates a signaling cascade without a direct action on gene transcription (3).

In the last decades, the endothelium has been recognized as a fundamental component of the vascular system. Besides its important function of regulating blood pressure and coagulation, the endothelium has been shown to play a crucial role in the inflammatory response (4). Leukocyte adhesion is known to be of great importance in this response. In inflammatory events, endogenous proinflammatory agents, like TNF-α, activate the resting endothelium and lead to an increased expression of endothelial cell adhesion molecules, which are responsible for the adhesion of leukocytes to the endothelium, resulting in leukocyte

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infiltration of inflamed tissue. Since we were interested in effects of Dex on inflammatory processes in the human endothelium, we used TNF-α-activated human umbilical vein endothelial cell (HUVEC) as a model of endothelial inflammation. To judge the anti-inflammatory effects of Dex, E-selectin, an important endothelial adhesion molecule (5), was chosen as inflammatory read-out parameter.

The involvement of kinases, such as MAPK, in the regulation of inflammatory processes has been intensively studied. The role of phosphatases, however, has as yet been very poorly investigated. Interestingly, recent reports show that MAPK phosphatases are important for the control of innate immune response processes (6, 7). We hypothesized that MAPK phosphatases could represent important targets for the novel "nontranscriptional" anti-inflammatory actions of glucocorticoids in endothelial cells. Moreover, we assumed that investigations into these mechanisms could lead to the identification of novel anti-inflammatory drug targets, aiming to improve anti-inflammatory therapy, to diminish adverse effects, or to overcome glucocorticoid resistance.

MATERIALS AND METHODS

HUVEC culture

Cells were prepared by digestion of umbilical veins with 0.1 g/l collagenase A (Roche, Mannheim, Germany) as described previously (8) and cultured in endothelial cell growth medium (Promocell, Heidelberg, Germany) containing 10% FCS (Biochrom, Berlin, Germany). Cells were routinely tested for mycoplasma contamination with the polymerase chain reaction (PCR) detection kit VenorGeM (Minerva Biolabs, Berlin, Germany). Cells were used at passage 3. Before treatment, confluent cells were starved overnight in a steroid-free medium consisting of phenol red-free Dulbecco's modified Eagle's medium (DMEM; Bio-Whittaker, Walkersville, MD) supplemented with 20% charcoal-stripped FCS.

Embryonic stem cell culture and differentiation

Mouse MAPK phosphatase-1 (MKP-1)$^{-/-}$ embryonic stem (ES) cells (9) were kindly provided by the Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ, USA). ES cells were thawed on mouse embryonic feeder cells and cultured in DMEM (Invitrogen, Carlsbad, CA, USA) containing 15% FBS (PAN Biotech, Aidenbach, Germany), 20 mM HEPES (Invitrogen), 1× MEM nonessential amino acids (Invitrogen), 0.1 mM β-mercaptoethanol (Invitrogen), and 1,500 U/ml leukemia inhibitory factor (LIF; Chemicon, Temecula, CA, USA). To remove feeder cells, ES cells were passaged for 2 wk on gelatin-coated dishes. Feeder cell-free MKP-1$^{-/-}$ and D3 ES cells (10) were cultivated in medium containing Glasgow MEM (Invitrogen), 10% FBS, 1× Na-pyruvate (Sigma-Aldrich, St. Louis, MO), 1× MEM nonessential amino acids, 0.05 mM β-mercaptoethanol, and 1,000 U/ml LIF. OP9 stroma cells (11) were cultured in α-MEM (Invitrogen) and 20% FBS. For ES cell differentiation, D3 and MKP-1$^{-/-}$ ES cells were cocultured with OP9 cells in differentiation medium (α-MEM, 10% FBS, and 0.1 mM β-mercaptoethanol); 72,000 D3 and MKP-1$^{-/-}$ cells were seeded on a 60 cm^2 dish containing confluent OP9 cells and incubated for 4 d before dissociation with Hank's enzyme-free dissociation buffer (Invitrogen). Cells were stained with a monoclonal allophycocyanin-linked anti-FLK1 antibody (Ab; ref 12) and propidium iodide (PI, Sigma-Aldrich). FLK$^+$PI$^-$ mesodermal cells were sorted with FACSAria (Becton Dickinson). After sorting, viable cells were counted and 31,000 D3 and MKP-1$^{-/-}$ cells were seeded in each well of a type IV collagen-coated 24-well plate (Becton-Dickinson, Franklin Lakes, NJ), respectively, and cultured in differentiation medium supplemented with 50 ng/ml vascular endothelial growth factor (VEGF; Sigma-Aldrich) for 4 days.

Determination of MKP-1 and (phospho-)p38 MAPK protein levels by Western blot analysis

Unless otherwise noted, HUVEC were treated with dexamethasone (Calbiochem) (1 nM, 60 min), TNF-α (Calbiochem, San Diego, CA, USA; 10 ng/ml, 15 min), SB203580 (Calbiochem; 10 μM, 60 min), RU486 (Calbiochem; 10 nM, 15 min), and sodium vanadate (Na_3VO_4; 100 μM, 30 min). Western blot analysis was performed as described previously (13). Antibodies used were as follows: rabbit polyclonal anti-MKP-1 (Santa Cruz Biotechnology, Santa Cruz, CA), antiphospho-p38 MAPK (Thr180/Tyr182; Cell Signaling), antip38 MAPK (Cell Signaling, Danvers, MA, USA), and horseradish peroxidase-conjugated goat anti-rabbit (Dianova, Hamburg, Germany). The Kodak 1D 3.5.4 software (Eastman Kodak, Stuttgart, Germany) was used for densitometric analysis.

Analysis of NF-κB p65 translocation by immunocytochemistry and confocal laser scanning microscopy

HUVEC were cultured on collagen (Biochrom)-coated glass cover slips, pretreated with dexamethasone (60 min) and treated with TNF-α (10 ng/ml, 30 min), fixed with 4% buffered formaldehyde (Sigma-Aldrich), permeabilized with 0.2% Triton X-100 (Sigma-Aldrich), and incubated with rabbit polyclonal anti-NF-κB p65 Ab (Santa Cruz) and Alexa Fluor 488-linked goat anti-rabbit Ab (Molecular Probes, Eugene, OR). A Zeiss LSM 510 Meta confocal lascer scanning microscope was used. For quantification of nucleic NF-κB p65 levels, the Scion Image software alpha 4.0.3.2 (Scion Corp, Frederick, MD, USA) was used.

Analysis of NF-κB DNA-binding activity by EMSA

HUVEC were treated with dexamethasone (60 min), pyrrolidine dithiocarbamate (50 μM, 60 min), parthenolide (5 μM, 60 min), and TNF-α (10 ng/ml, 60 min). Nuclear protein extracts were prepared, and electrophoretic mobility shift assays were performed as described previously (13). Bands were visualized by the Cyclone Storage Phosphor System (Canberra-Packard, Dreieich, Germany).

Determination of MKP-1 mRNA levels by quantitative reverse transcriptase-PCR

Total mRNA was extracted with the RNeasy mini kit (Qiagen, Hilden, Germany) and M-MuLV reverse transcriptase (New England BioLabs, Beverly, MA) was used. Real-time PCR was performed with AmpliTaq Gold (Roche). Human MKP-1 primers (biomers.net, Ulm, Germany): 5′-GACGCTCCTCTCTCAGTCCAA-3′ (forward); 5′-GGCGCTTTTCGAGGAAAAG-3′ (reverse); 5′-TTCGGCGCAGAGAGACCCGG-3′ (probe). Results were quantified based on the relative expres-

sion of the MKP-1 gene *vs.* the housekeeping gene GAPDH using the model of Pfaffl (14).

Analysis of MKP-1 exon 2 integrity by PCR

Genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen). PCR was performed with the DyNAmo Probe qPCR Kit (Finnzymes, Espoo, Finland). Mouse MKP-1 primer (biomers.net): 5′-CAGGTACTGTGTCGGTGGTG-3′ (forward); 5′-CCTGGCACAATCCTCCTAGA-3′ (reverse). Bands of PCR products were visualized on a Kodak 440cf image station.

MKP-1 antisense experiments

For transfection of human MKP-1 antisense (5′-ggtccCGAATGTGCTGagttc-3′) and sense phosphorothioate oligonucleotides (biomers.net), jetPEI (Polyplus-Transfection, San Marcos, CA, USA) was used. Further experiments were started 24 h after transfection.

Analysis of E-selectin levels by flow cytometry

Cells were pretreated with dexamethasone (60 min) or SB203580 (10 μM, 60 min), treated with TNF-α (10 ng/ml, 4 h), detached by trypsinization, incubated in 4% buffered formaldehyde solution (Sigma-Aldrich), and stained with R-phycoerythrin-linked mouse monoclonal anti-human CD62E Ab (Leinco, St. Louis, MO, USA) or rat monoclonal anti-mouse CD62E Ab (Abcam, Cambridge, UK) and Alexa Fluor 488-linked goat antirat Ab (Molecular Probes). Flow cytometric analysis was performed with FACSCalibur (Becton Dickinson).

Statistical analysis

The number of independently performed experiments is stated in the respective figure legend. One representative image is shown. Bar graph data are mean ± SE. Statistical analysis was performed with the GraphPad Prism software version 3.03 (GraphPad Software, San Diego, CA, USA). Unpaired *t* test was used to compare two groups. To compare three or more groups, one-way ANOVA followed by Newman-Keuls post hoc test was used.

RESULTS

Dex at low concentrations reduces TNF-α-induced E-selectin expression and does not influence NF-κB

Dex reduces TNF-α-induced E-selectin expression in endothelial cells (15). We confirmed this fact in our

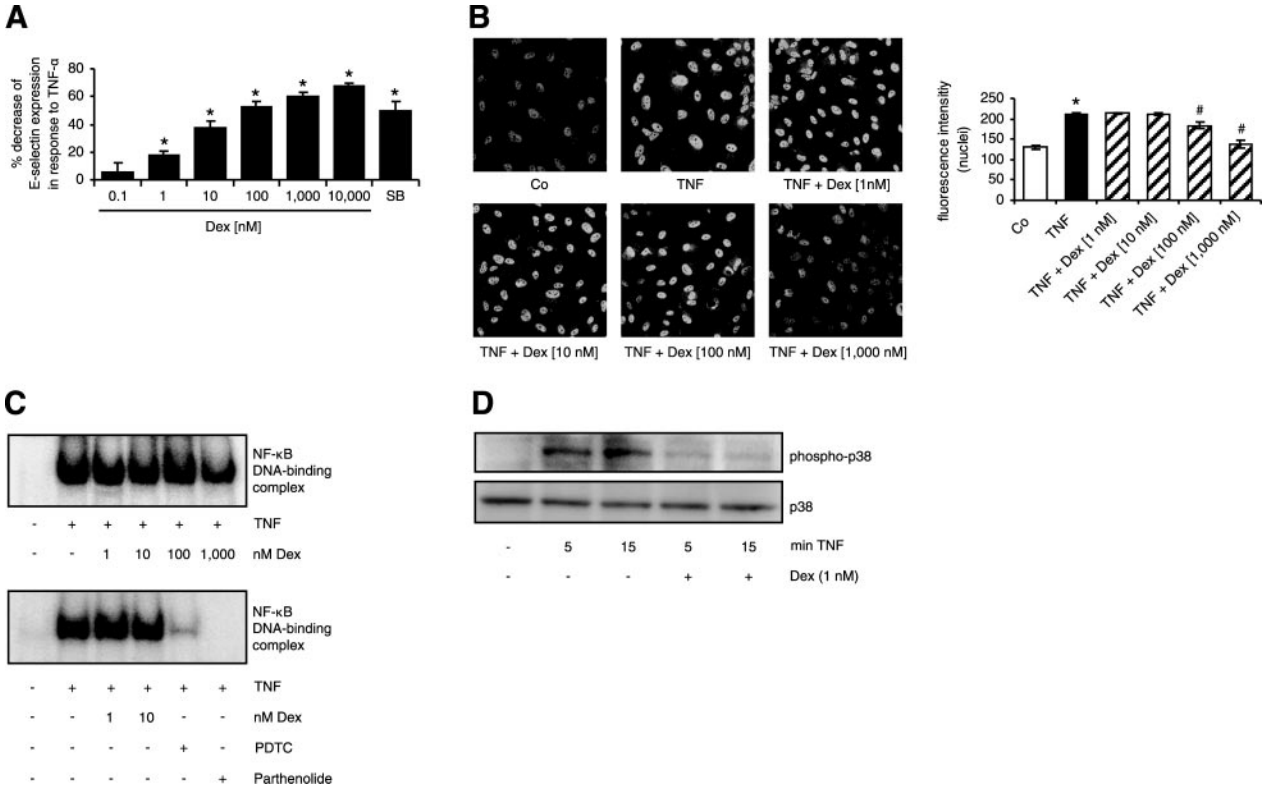

Figure 1. Dex at low concentrations readily decreases TNF-α-induced E-selectin expression and p38 MAPK activity, but does not influence NF-κB. *A*) Treatment of HUVEC with dexamethasone leads to a concentration-dependent decrease of E-selectin expression in response to TNF-α. A p38 MAPK inhibitor (SB203580) was also able to decrease E-selectin expression on TNF-α-treatment. *, $P \leq 0.05$ *vs.* untreated control ($n=3$). *B*) left panel: TNF-α increases amount of NF-κB p65 subunit in nucleus. Dex at low concentrations (1–100 nM) does not influence this effect, whereas a higher Dex concentration (1,000 nM) decreases the amount of NF-κB p65 subunit ($n=2$). *B*) right panel: Quantitative analysis of nucleic NF-κB p65 content. *$P \leq 0.05$ *vs.* untreated control. #$P \leq 0.05$ *vs.* only TNF-α-treated. *C*) TNF-α-induced NF-κB DNA-binding activity is not affected by Dex at low concentrations (1–100 nM), whereas a higher Dex concentration (1,000 nM) and the well-known NF-κB inhibitors pyrrolidine dithiocarbamate (PDTC) and parthenolide diminish TNF-α-evoked NF-κB DNA-binding activity ($n=4$). *D*) Treatment with TNF-α leads to a phosphorylation (*i.e.*, activation) of p38 MAPK. Dex at a low concentration (1 nM) abrogates this effect ($n=4$).

cell system: pretreatment with Dex concentration dependently reduced the expression of E-selectin (**Fig. 1*A***). Importantly, even low concentrations of Dex (1–100 nM) lead to a highly significant reduction of E-selectin expression. The transcription factor NF-κB is thought to play a key role in the up-regulation of E-selectin on TNF-α treatment (16) and Dex is known to interfere with the NF-κB pathway (17). However, we found that Dex at low concentrations readily decreases TNF-α-induced E-selectin expression, whereas it does not influence TNF-α-activated NF-κB: Dex (1–10 nM) neither alters the TNF-α-evoked translocation of the NF-κB p65 subunit (Fig. 1*B*) nor the increase of NF-κB DNA-binding activity (Fig. 1*C*). Only high Dex concentrations (≥1,000 nM) were able to abolish the TNF-α-induced NF-κB activity (Fig. 1*C*), as did the known NF-κB inhibitors pyrrolidine dithiocarbamate and parthenolide. For full activation of TNF-α-induced E-selectin gene transcription, besides NF-κB activation, the induction of p38 MAPK has been reported to be of importance (18). Therefore, we treated human endothelial cells with a pharmacological inhibitor of p38 MAPK activity (SB203580), which in fact led to a strong decrease of E-selectin expression in response to TNF-α (Fig. 1*A*). Importantly, TNF-α-evoked p38 MAPK activity is abrogated by Dex at low concentrations (1 nM; Fig. 1*D*), pointing to a crucial role for p38 MAPK in the up-regulation of E-selectin in our system.

Dex decreases p38 MAPK activity via an induction of MKP-1

We assumed that Dex could activate a MAPK phosphatase, which accounts for the decrease of p38 MAPK activity. In fact, on treatment of HUVEC with sodium orthovanadate, an inhibitor of phosphatases, we observed a strongly diminished influence of Dex on TNF-α-induced p38 MAPK activity (**Fig. 2*A***), suggesting that phosphatases are indeed involved. Furthermore, we hypothesized that MKP-1, the archetypal member of the family of dual specificity MAPK phosphatases, is up-regulated by Dex. In fact, we found that Dex concentration (Fig. 2*B*) and time dependently (Fig. 2*C*) leads to an increase in endothelial MKP-1 protein levels. Maximum MKP-1 protein levels were detected after ~60 min treatment with 1 nM Dex. Analysis of MKP-1 mRNA levels by quantitative RT-PCR showed a rapid increase of MKP-1 mRNA on Dex treatment within 5 min (Fig. 2*D*). Moreover, this up-regulation of MKP-1 depends on the activation of the glucocorticoid receptor, as shown be the use of the glucocorticoid

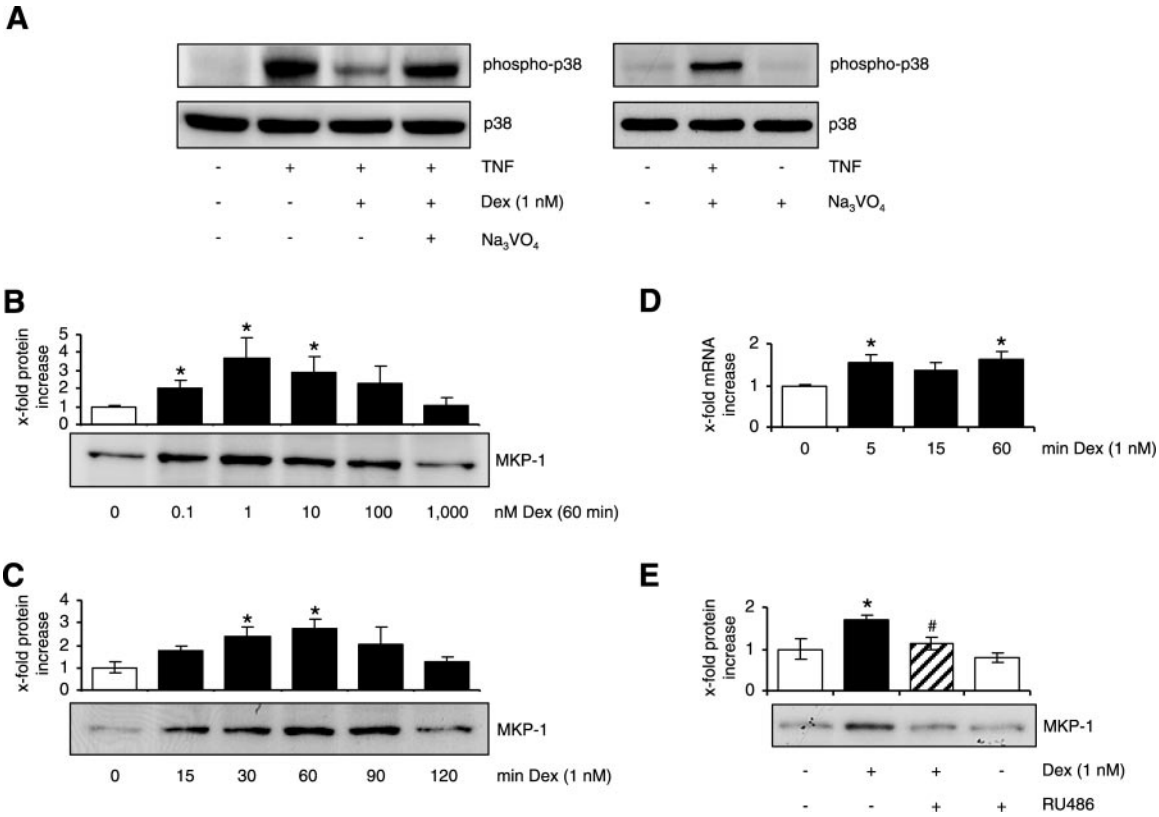

Figure 2. Dex at low concentrations up-regulates endothelial MKP-1 expression. *A*) left panel: Treatment of HUVEC with Dex attenuates the TNF-α-induced phosphorylation (*i.e.*, activation) of p38 MAPK. The phosphatase inhibitor sodium vanadate (Na_3VO_4) reverses this effect (n=4). *A*) right panel: (control experiments): Na_3VO_4 does neither alter basal nor TNF-α-induced p38 MAPK phosphorylation (*i.e.*, activation) (n=3). *B*) Dex concentration dependently increases expression of MKP-1 protein (n=7). *C*) Dex time-dependently increases expression of MKP-1 protein (n=5). *D*) Dex induces the expression of MKP-1 mRNA (n=3). *E*) The glucocorticoid receptor antagonist RU486 is able to block induction of MKP-1 protein by Dex (n=3). $^{*}P \leq 0.05$ *vs.* untreated control. $^{\#}P \leq 0.05$ *vs.* only Dex treated.

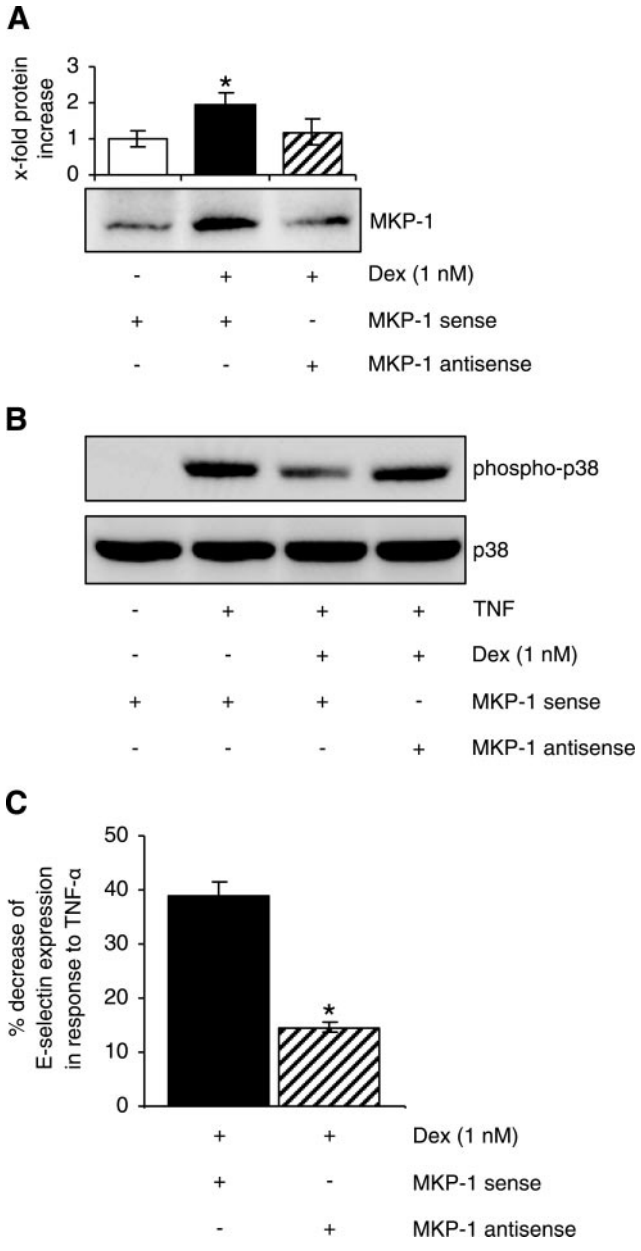

Figure 3. MKP-1 antisense oligonucleotides block the effect of Dex on p38 MAPK activity and E-selectin expression. *A*) MKP-1 antisense oligonucleotides prevent the induction of MKP-1 protein by Dex. $*P \leq 0.05$ *vs.* only MKP-1 sense treated ($n=3$). *B*) Dex attenuates TNF-α-induced phosphorylation (*i.e.*, activation) of p38 MAPK. In MKP-1-silenced (antisense treated) cells, this ability of Dex is abolished. *C*) Dex decreases the TNF-α-evoked expression of E-selectin. In MKP-1-silenced (antisense treated) cells, this effect of Dex is diminished. $*P \leq 0.05$ *vs.* Dex + MKP-1 sense-treatment ($n=4$).

receptor antagonist RU486, which blocked the induction of MKP-1 protein by Dex (Fig. 2*E*).

MKP-1 antisense restores p38 MAPK activation and E-selectin expression

To clarify whether MKP-1 plays a causal role in the reduction of TNF-α-induced p38 MAPK activation and E-selectin expression, we used an MKP-1 antisense approach. First, we confirmed the functionality of the applied MKP-1 antisense oligonucleotides: **Fig. 3*A*** shows that transfection of HUVEC with MKP-1 antisense but not sense oligonucleotides abolished cellular MKP-1 levels increased by Dex. We then treated MKP-1-silenced endothelial cells with Dex and TNF-α and found that the capability of Dex to diminish the TNF-α-induced activation of p38 MAPK (Fig. 3*B*) and E-selectin expression (Fig. 3*C*) was significantly diminished. This points to a crucial involvement of MKP-1 in the deactivation of p38 MAPK and reduction of E-selectin expression by Dex.

MKP-1 knockout endothelial cells do not react to Dex treatment

In addition to the antisense approach, we used an MKP-1 knockout approach. MKP-$1^{-/-}$ mouse ES cells (9) were differentiated into endothelial cells. To confirm the lack of MKP-1 gene in ES cells, we checked the integrity of MKP-1 exon 2: wild-type (WT) ES cells show an intact exon 2, whereas exon 2 of knockout cells is disrupted (9; **Fig. 4*A***). In MKP-1 WT endothelial cells, Dex led to a clear inhibition of E-selectin expression on TNF-α treatment. $MKP^{-/-}$ endothelial cells, however,

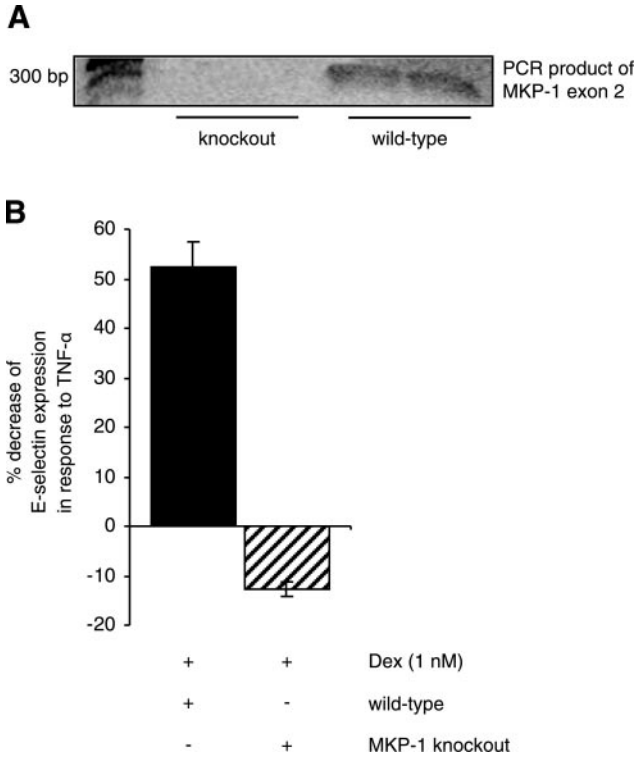

Figure 4. Effect of Dex on E-selectin expression is prevented in endothelial MKP-1 knockout cells. *A*) Embryonic stem cells from MKP-1 WT and knockout mice were differentiated into endothelial cells. In knockout cells, exon 2 of MKP-1 gene is destroyed. WT cells show an intact exon 2. *B*) Dex leads to a decrease of E-selectin expression in response to TNF-α treatment. In MKP-1 knockout endothelial cells, this effect is abrogated.

did not respond to Dex (1 nM): these cells even show a slight increase of E-selectin expression (Fig. 4*B*).

DISCUSSION

We found that Dex at very low concentrations (1–100 nM) leads to a highly significant reduction of E-selectin induced by TNF-α. It is commonly accepted that the up-regulation of E-selectin in activated endothelial cells is an NF-κB-regulated event (16) and that Dex interferes with the NF-κB pathway (17). This interference is thought to be one of the major mechanisms of action of Dex responsible for its anti-inflammatory properties. Therefore, it was intriguing to find that Dex at low concentrations readily decreases TNF-α-evoked E-selectin expression, whereas it does not influence TNF-α-activated NF-κB.

Besides the known NF-κB-dependent up-regulation of E-selectin by TNF-α, an additional pathway has been described that occurs in parallel to the NF-κB-pathway: TNF-α activates both p38 MAPK and c-Jun NH2-terminal kinase (JNK), resulting in the phosphorylation of the transcription factors activating transcription factor-2 (ATF-2) and c-Jun, respectively. The NF-κB- and the ATF-2/c-Jun-pathway converges on the E-selectin promoter. Both pathways are required for full activation of E-selectin gene transcription in response to TNF-α (18). Moreover, a study by Reimold *et al.* stressed the importance of ATF-2 activation for E-selectin expression, since ATF-2-deficient mice show an attenuated E-selectin expression (19). These facts highlight the important role for p38 MAPK in the up-regulation of E-selectin by TNF-α. In accordance with these findings, we found that a pharmacological inhibitor of p38 MAPK activity was able to significantly decrease E-selectin expression (~50% reduction) in HUVEC.

In recent years, an influence of glucocorticoids on different members of the MAPK family has been increasingly recognized as a novel mechanism involved in glucocorticoid action (20). In line with this new concept, we found that pretreatment of Dex (1 nM) abrogates TNF-α-induced p38 MAPK activity in human umbilical endothelial cells. A study by Pelaia *et al.* reporting that Dex (100 nM) is able to prevent TNF-α-induced activation of p38 MAPK in human pulmonary endothelial cells (21) supports our results. Interestingly, González *et al.* revealed that TNF-α-activated p38 MAPK is not influenced by Dex at 1,000 nM in the human endothelial cell line HMEC-1 (22). These facts suggest a concentration-dependent effect of Dex on the activation of p38 MAPK.

The question arose how Dex is able to decrease the activity of p38 MAPK. Our assumption that a phosphatase could account for this decrease was confirmed by the fact that a phosphatase inhibitor blocked the effect of Dex on p38 MAPK. We have shown MKP-1 to be crucially involved in the deactivation of TNF-α-induced p38 MAPK in endothelial cells (8, 23). Additionally, Lasa *et al.* reported that MKP-1 is the only p38 MAPK-inhibiting phosphatase induced by Dex in epithelial cells (24). Therefore, we hypothesized an induction of MKP-1 by Dex and, in fact, found a time- and concentration-dependent up-regulation of MKP-1. We for the first time provide evidence that a glucocorticoid is able to increase MKP-1 protein levels in the human endothelium. Analysis of the MKP-1 mRNA level revealed that it raises within 5 min, which easily explains the rapid induction of MKP-1 protein within 15 min. Employing an inhibitor of the glucocorticoid receptor, we proved an involvement of this receptor in the induction of MKP-1 by Dex, which excludes an unspecific effect of Dex by binding to other subcellular structures than the glucocorticoid receptor.

It has to be mentioned that in most studies Dex is used at concentrations of ~100 nM, whereas in our system Dex concentrations as low as 1 nM lead to a strong up-regulation of MKP-1 and an effective deactivation of p38 MAPK. Interestingly, higher Dex concentrations (≥1,000 nM) do not lead to an induction of MKP-1 but reduce NF-κB activity. This suggests that the pathways Dex uses for the transduction of its anti-inflammatory properties depend on the applied concentration. It can be speculated that low glucocorticoid concentrations, which are therapeutically preferred to limit side effects, exert their anti-inflammatory potential in endothelial cells rather via induction of MKP-1 than via blockade of NF-κB.

An involvement of MKP-1 in the action of glucocorticoids in endothelial cells has as yet been hypothetical (20). By using an MKP-1 antisense and knockout approach, we for the first time provide evidence that Dex-induced MKP-1 plays a pivotal role in the reduction of p38 MAPK and, most importantly, E-selectin expression induced by TNF-α .

In summary, our study highlights the relevance of MKP-1 as an important player in inflammatory reactions of the human endothelium. MKP-1 represents a novel and crucial mediator of the anti-inflammatory actions of glucocorticoids at low concentrations. The pathways glucocorticoids use for their anti-inflammatory action obviously depend on the applied glucocorticoid concentration. In conclusion, the present study points out the importance of studying mechanisms of action of well-established anti-inflammatory pharmaceuticals, since this approach could lead to re-evaluation of anti-inflammatory principles: our data indicate that MKP-1 represents a promising pharmacological target for the treatment of inflammatory diseases. FJ

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