Respiratory Medicine (2005) **99**, 1068–1069

respiratoryMEDICINE

LETTER TO THE EDITOR

PDTC and NO suppress CC chemokine production in TNF-α-stimulated human pulmonary microvascular endothelial cells

Dear Sir,

We recently reported in this journal that the expression of adhesion molecules, including E-selectin, ICAM-1 and VCAM-1, in human pulmonary microvascular endothelial cells (PMVEC) induced by TNF-α is inhibited significantly by pretreatment with pyrrolidine dithiocarbamate (PDTC) or spermine NONOate (Sper-NO), possibly in part through blocking the activation of NF-κB.[1] As demonstrated by Blease et al. and others,[2,3] human PMVEC are capable of synthesizing chemokines such as IL-8, monocyte chemotactic protein (MCP)-1 and regulated upon activation, normal T-cell expressed and secreted (RANTES) upon a variety of pro-inflammatory stimuli. However, redox-regulated mechanisms of chemokine production in human PMVEC have never been elucidated. We therefore evaluated the impact of PDTC and Sper-NO on the production of CC chemokines induced by TNF-α in human PMVEC.

Human PMVEC were cultured as described recently.[1] Cell suspensions (5×10^4 cells per well) were seeded on 24-well culture clusters. After cells reached confluence, human PMVEC were incubated for 8 h in M199 medium alone or with 10 ng/ml TNF-α in a final volume of 300 μl. In other wells, cells were pretreated for 1 h in M199 medium with 0.1 mM PDTC or 1 mM Sper-NO; they were then incubated in the same medium alone or with 10 ng/ml TNF-α for 8 h. Cell-free supernatants from resting or stimulated human PMVEC cultures were tested for MCP-1, RANTES (Biosource International,

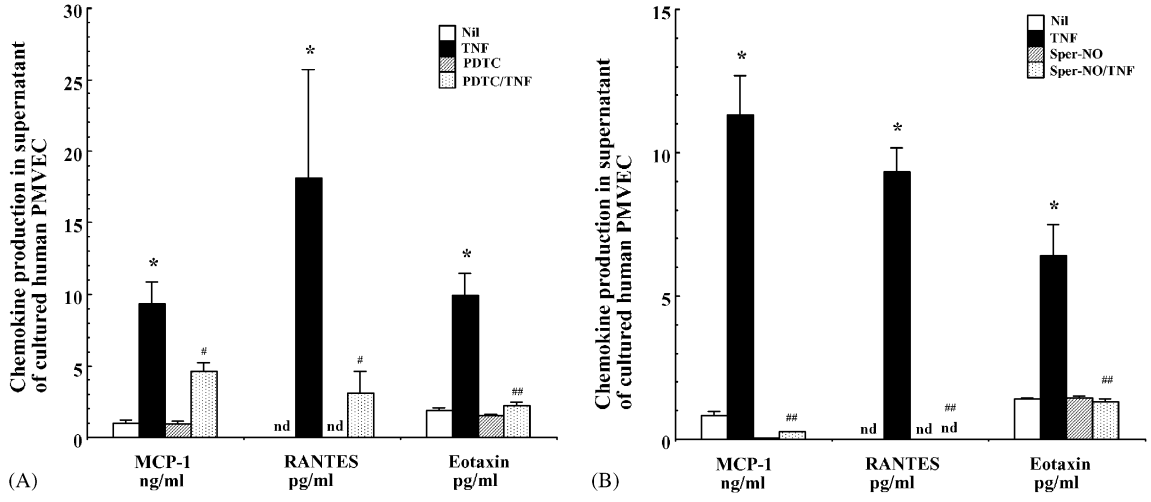

Figure 1 Effects of PDTC (A) and Sper-NO (B) on the production of MCP-1, RANTES and eotaxin in TNF-α-stimulated human PMVEC. Cells were without pretreatment or pretreated with 0.1 mM PDTC or 1 mM Sper-NO for 1 h; thereafter, they were not stimulated or stimulated with TNF-α (10 ng/ml) for 8 h. The Nil groups consisted of cells incubated with medium alone. Quadruplicate supernatants for each condition were harvested, and ELISA measured the levels of MCP-1, RANTES and eotaxin. Results were expressed as the mean ± SEM of six independent experiments. Differences between groups were examined using the paired *t*-test. *P*-values <0.05 were considered statistically significant. The minimal detection limit of MCP-1, RANTES and eotaxin was 20, 5 and 3 pg/ml, respectively. An arbitrary value of half of the minimal detection limit was used for data analysis, when "non-detectable" results were obtained. $^{*}P<0.001$ vs. Nil; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. TNF-α alone. nd: not detectable.

0954-6111/$ - see front matter
doi:10.1016/j.rmed.2005.01.011

Inc., CA) and eotaxin (R&D Systems, Inc., MN) contents by sandwich ELISA kits according to the manufacturers' protocols.

Figure 1 shows that treatment of human PMVEC with TNF-α for 8 h resulted in a significant increase in the production of MCP-1, RANTES and eotaxin, as compared to the non-stimulated cells. Of note, pretreatment for 1 h with PDTC or Sper-NO significantly inhibited the production of MCP-1, RANTES and eotaxin induced by TNF-α.

Chemokines are divided into the CXC, CC, C and CX3C subfamilies. Of these, CC chemokines are most diversified and act on monocytes, activated T-cells, eosinophils and basophils.[4] The NF-κB plays an important role in the gene regulation of chemokines, including MCP-1, RANTES and eotaxin. As shown in our recent paper, PDTC and Sper-NO inhibit NF-κB activation in human PMVEC.[1] It was reported that PDTC inhibits the production of IL-8, MCP-1 and RANTES in human umbilical vein endothelial cells and pancreatic periacinar myofibroblasts through a blockade of NF-κB activation.[5,6] NO suppresses the production of MCP-1 and RANTES in human umbilical vein endothelial cells and keratinocyte cell line HaCaT.[7,8]

Our recent[1] and present findings indicate that both adhesion molecule expression and chemokine production in human PMVEC induced by TNF-α are inhibited significantly by pretreatment with PDTC or Sper-NO, possibly via blocking redox-regulated NF-κB activation. These results suggest that restoration of the redox balance using antioxidants or NO may offer potential therapeutic approach in cytokine-mediated inflammatory reactions in the human lung.

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