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Does TNF-α directly increase endothelial cell monolayer permeability?

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Abstract

The effects of dexamethasone (DEX) and $N\varpi$-nitro-L-arginine methyl ester (L-NAME) on the tumour necrosis factor-α (TNF-α)-induced increase in permeability of human umbilical vein endothelial cell (HUVEC) monolayer to [^{125}I] labelled bovine serum albumin (BSA) were examined. Preincubation of HUVEC monolayers with DEX (1 μ*M*, 2 h) completely abolished the effect of TNF-α (5 ng/ml, 18 h). Administration of DEX 2 h after TNF-α also reduced the effect of TNF-α, while L-NAME (5 ng/ml, 1 m*M*, 18 h) had no significant effect.

The observed inhibition of the TNF-α-induced permeability increase on preincubation with DEX would suggest a role for nitric oxide (NO) in mediating the permeability response. However, this is not confirmed by the experiments with L-NAME. The inhibition caused by DEX administered after TNF-α would suggest alternative mechanisms by which DEX may be acting in addition to inhibition of NO synthase induction.

Introduction

Tumour necrosis factor-α (TNF-α) is believed to play a central role in the pathogenesis of cerebral oedema associated with bacterial meningitis [1]. There is also increasing evidence that TNF-α is one of the primary cytokines responsible for many of the changes associated with life-threatening sepsis, including hypotension, poor peripheral perfusion, oedema and subsequent respiratory and cardiac failure (reviewed in [2]). Using an *in vitro* model system, cultured human umbilical vein endothelial cells (HUVEC) grown on Transwell-COL membrane assemblies, we have previously shown [3] that TNF-α increased HUVEC monolayer permeability to [^{125}I]-labelled bovine serum albumin (BSA) in a time- and dose-dependent manner. We have also shown that this effect was inhibited by a TNF-α-neutralizing antibody, and was not due to contamination by endotoxin. In the present study we used the same model system to investigate the effect of dexamethasone (DEX) (an inhibitor of nitric oxide (NO) synthase induction) and $N\varpi$-nitro-L-arginine methyl ester (L-NAME) (a competitive inhibitor of NO synthase) on the TNF-α-induced increase in permeability, since TNF-α stimulation of endothelial cells (EC) has been shown to induce the release of NO [4]. The purpose of this study, therefore, was to determine whether TNF-α affects HUVEC monolayer permeability directly or whether its effect is due to induction of NO.

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Materials and methods

Human recombinant TNF-α (specific activity $>2.0 \times 10^7$ U/mg) was obtained from Boehringer Mannheim. L-NAME and DEX were obtained from Sigma and [^{125}I]-labelled bovine serum albumin (BSA) (specific activity = 0.95 mCi/mg) from ICN Radiochemicals. HUVEC were isolated from umbilical vein by collagenase digestion according to the method of Jaffe et al. [5] and grown to confluence (3–4 days) in medium E199 + 20% foetal bovine serum (FBS) + EC growth factors. Confluent cells were trypsinized (0.05% trypsin + 0.02% EDTA) and seeded at a density of 6×10^5 cells/cm^2 onto Transwell-COL membrane assemblies (6.5 mm diameter, 0.4 μ*M* pore size, collagen-treated transparent membrane; Costar), suspended in 24-well culture plates. The membranes were incubated with 25 μg/ml fibronectin for 3 h before use. Two days after seeding, the monolayers were treated with TNF-α alone or in the presence of DEX (1 μ*M*) or L-NAME (1 mM). Permeability was determined by incubating monolayers with [^{125}I]-labelled BSA in RPMI 1640 + 10% FBS. [^{125}I]-labelled BSA (2.5×10^5 cpm/100 μl) was added to the upper chamber of the membrane assembly and sampled from the lower after incubation at 37 °C for 1 h in a shaking water bath (10 rpm). Radioactivity was determined by γ counting, and the results expressed as percentage clearance (counts detected in the lower chamber after 60 min expressed as a percentage of total counts added to upper chamber). The percentage clearance was used as a measure of permeability. Data are presented as mean ± SD of *n* observations. Statistical analysis was performed using the Mann–Whitney–Wilcoxon test. Differences were deemed significant if $p < 0.05$.

Results and discussion

Preincubation of HUVEC monolayers with DEX (1 μ*M*, 2 h) completely abolished the effect of TNF-α (5 ng/ml, 18 h) (Fig. 1A). Administration of DEX (1 μm) 2 h after TNF-α (5 ng/ml) significantly ($p < 0.01$) reduced clearance (4.46 ± 0.51%) compared to the effect of TNF-α alone (8.27 ± 0.46%) at 18 h, but was significantly greater ($p < 0.05$) than the control (3.48 ± 0.37%) (Fig. 1B). Clearance of [^{125}I] BSA induced by TNF-α + L-NAME (5 ng/ml, 1 mM, 18 h) was 7.68 ± 0.28% which was not significantly different ($p > 0.05$) from that produced by TNF-α alone (8.27 ± 0.45%) (Fig. 2).

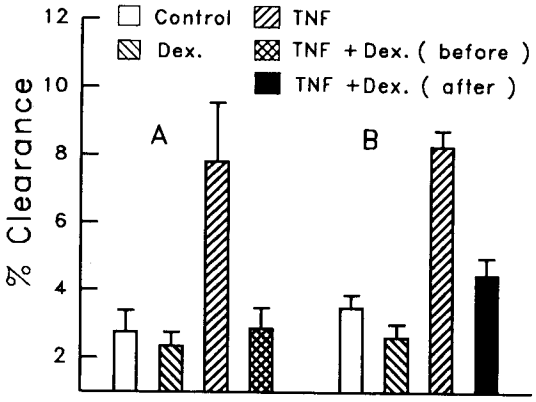

Figure 1
Percentage clearance of counts across HUVEC monolayers after incubation for 18 h with TNF-α (5 ng/ml) alone and in the presence of DEX (1 μ*M*). (2 h preincubation (A) or added 2 h after TNF-α (B)). Results are means ± SD of 4–6 observations.

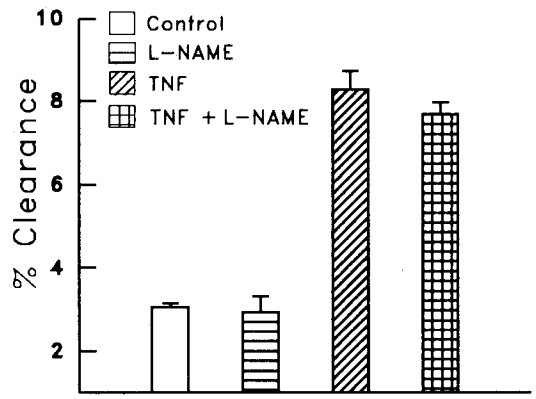

Figure 2
Percentage clearance of counts across HUVEC monolayers after incubation for 18 h with TNF-α (5 ng/ml) alone and in the presence of L-NAME (1 m*M*). Results are means ± SD of 4–6 observations.

The observed inhibition of the TNF-α-induced permeability increase by preincubation with DEX would suggest a role for NO in mediating the permeability response. However, this is not confirmed by the experiments carried out with L-NAME. Administration of DEX 2 h after TNF-α also caused a significant inhibition of the permeability effect. This would suggest that DEX may be acting by other mechanisms in addition to a possible inhibition of NO synthase induction. Therefore, TNF-α may have a direct effect on HUVEC

monolayer permeability, although an involvement of NO cannot be completely ruled out.

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