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IL-4 inhibits LPS-, IL-1β- and TNFα-induced expression of tissue factor in endothelial cells and monocytes

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Inflammatory mediators such as endotoxin, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) dose-dependently increased the expression of tissue factor on the surface of cultured bovine aortic endothelial cells (ABAE), human umbilical vein endothelial cells (HUVEC) and human monocytes. In ABAE, endotoxin-, IL-1β- and TNFα-induced tissue factor expression was suppressed by interleukin-4 (IL-4) which also neutralized the pyrogenic effect of endotoxin in HUVEC and monocytes. IL-4 did not alter TNF-α-induced procoagulant changes in HUVEC and monocytes but strongly protected the monocyte surface against IL-1β-induced procoagulant changes.

1. INTRODUCTION

Tissue factor (TF) is an ubiquitous membrane-anchored glycoprotein that initiates blood coagulation by forming a complex with circulating factors VII and VIIa [1]. Under normal circumstances, endothelial cells do not express TF activity while they constitutively express thrombomodulin (TM) which accelerates the thrombin-catalysed activation of protein C thus contributing to the anticoagulant properties of the endothelium. In some pathological situations, when the endothelium or the monocytes are exposed to inflammatory mediators, they can acquire procoagulant properties [2–4]. Indeed, stimulation of these cells by inflammatory compounds such as endotoxin, interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α) may alter the anti-thrombotic properties of the endothelium by inducing the expression of TF and the down-regulation of TM, therefore promoting coagulation and thrombosis [2–4]. Only a few compounds have been shown to reduce the effect of these inflammatory mediators [5] and IL-4 has been recently described as an inhibitor of pyrogen-induced down-regulation of TM in cultured human vascular endothelial cells [6].

We investigated the effect of IL-4 on the expression of TF induced by various inflammatory mediators on the surface of endothelial cells and monocytes.

2. MATERIALS AND METHODS

2.1. *Cells*

Adult bovine aortic endothelial cells (ABAE) (passage 7–10) were isolated and cultured as already described [7] in 96-well microplates in Dulbecco's modified Eagle medium, supplemented with 10% fetal calf serum and bovine basic fibroblast growth factor (1 ng/ml) (Amersham, France).

Human umbilical vein endothelial cells (HUVEC) (passage 3–10) were isolated and cultured as described [8] in 96-well microplates in F12-Ham's medium supplemented with 10% fetal calf serum, endothelial cell growth factor (30 μg/ml) and heparin (100 μg/ml) (Sigma, France).

Mononuclear cells were obtained from human heparinized blood as described by Boyum [9]. Cells were plated for 30 min at 37°C into 96-well microplates (10^5 cells/well). Non-adherent cells were then harvested and adherent monocyte (5×10^3 cells/well) were used for the assay.

2.2. *Determination of tissue factor activity on the cells*

Procoagulant activity was assayed according to Suprenant et al. [10]. Briefly, adherent cells were incubated at 37°C in M-199 (without Phenol red) with endotoxin (LPS-lipopolysaccharide from *E. coli* strain: 055:B5) (Sigma, France), IL-1β or TNF-α (Tebu, France) in the absence or presence of the indicated concentrations of IL-4 (Tebu, France). Incubation lasted for 18 h for ABAE and monocytes and 6 h for HUVEC. The medium was removed and wells were washed twice with 1 ml of phosphate-buffered saline (PBS) and incubated for 45 min at 37°C with 250 μl of M-199 containing PPSB (0.44 U/ml FVII) (Intertransfusion, France) and 100 μg/ml of substrate S2222 (Kabi, Sweden). The optical density (OD) was measured at 405 nm. The TF activity was obtained from a standard curve (log [ΔOD_{405}/min] vs. log [U/ml]) using serial dilutions of rabbit brain thromboplastin in M-199 assayed as described above. Undiluted thromboplastin was arbitrarily assigned a value of 1 U/ml. The TF activity was normalized to the cell counts from the same well and expressed as μU of TF/10^5 cells.

3. RESULTS AND DISCUSSION

3.1. *Effect of LPS, IL-1β and TNF-α on TF expression in ABAE, HUVEC and monocytes*

The addition of LPS, IL-1β or TNF-α to adherent cells resulted in a time-dependent expression of TF on the cell surface (Fig. 1). For both ABAE and monocytes, increased TF activity was observed as early as 4 h and reached a maximum 18 h after stimulation. in HUVEC, LPS, IL-1β or TNF-α induced rapid surface

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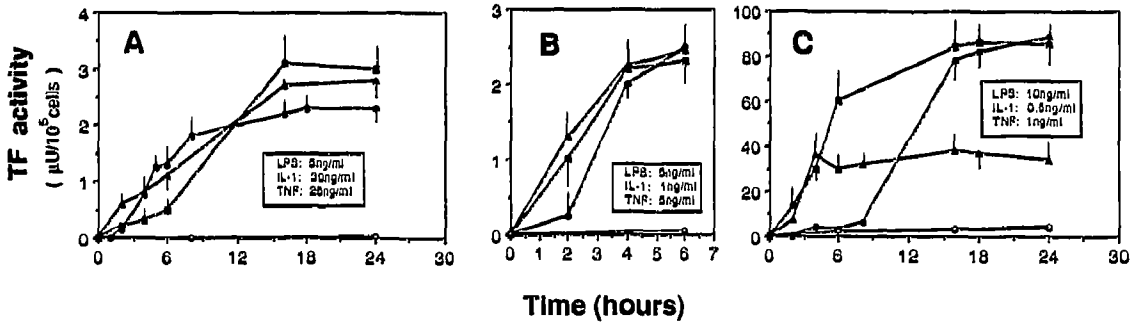

Fig. 1. Time course of LPS, IL-1β or TNF-α-stimulated TF expression in endothelial cells and monocytes. ABAE (A), HUVEC (B) and human monocytes (C) were incubated with the indicated concentrations of LPS (●), IL-1β (■), TNF-α (▲) or the vehicle (○). Results are expressed as mean ± S.D. (n = 6).

expression of TF which reached a maximum around 6 h. These results confirm previous observations showing that these compounds induce endothelial cell procoagulant activity while suppressing cell-surface anticoagulant activity [11,12]. For both ABAE and monocytes, TF expression in control wells did not vary significantly over the 24-h incubation period and the passage number had no effect on the procoagulant activity of the various inflammatory mediators (not shown).

Exposure of confluent ABAE or HUVEC and adherent monocytes to increasing concentrations of LPS, IL-1β or TNF-α resulted in a significant increase in TF expression on the cell surface (Fig. 2). LPS-stimulated expression of TF occurred in a dose-dependent manner, a maximal response being attained for the three cell types around 1 ng/ml. After incubation with the highest doses of LPS, human monocytes exhibited higher maximal levels of TF, compared to LPS-stimulated ABAE or HUVEC. Indeed, a ≈30-fold difference was observed between endothelial cells and monocytes. Such a difference between cell types was also observed after stimulation with IL-1β, monocytes being of a higher sensitivity than endothelial cells for IL-1β-mediated TF expression (Fig. 2C). In both endothelial cell types, TNF-α stimulated TF expression in a dose-dependent manner with a maximum effect around 10 ng/ml. In monocytes however, TFNα-induced TF expression was lower than that observed for LPS or IL-1β in these cells (Fig. 2C).

3.2. *Effect of IL-4 on pyrogen-induced TF expression in ABAE, HUVEC and monocytes*

As shown in Fig. 3A, IL-4 counteracted in a dose-dependent manner IL-1β-, TNFα- and LPS-induced TF induction in ABAE. However, IL-4 was not able to totally abolish pyrogen-induced TF expression, the maximum effect (about 60% inhibition) being attained around the dose of 1 ng/ml. The IC_{50} value (concentration which inhibited 50% of the pyrogen-induced TF expression) with regard to the effect of all inflammatory mediators was between 0.05 and 0.5 ng/ml.

In HUVEC, IL-4 strongly reduced LPS-induced TF expression with an IC_{50} value of 0.5 ng/ml. With regard to IL-1β or TNF-α, however, IL-4 did not show any inhibitory effect. This observation confirms the results published recently by Kapiotis et al. [6] showing no influence of IL-4 on pyrogen-induced TF expression in HUVEC but they raise a controversy with regard to the effect of LPS. One possible explanation for such a discrepancy may be the great difference in the amount of LPS used in both studies. Indeed, the 4000-fold lower LPS concentration used for optimal TF expression in our experimental conditions, may give higher sensitivity

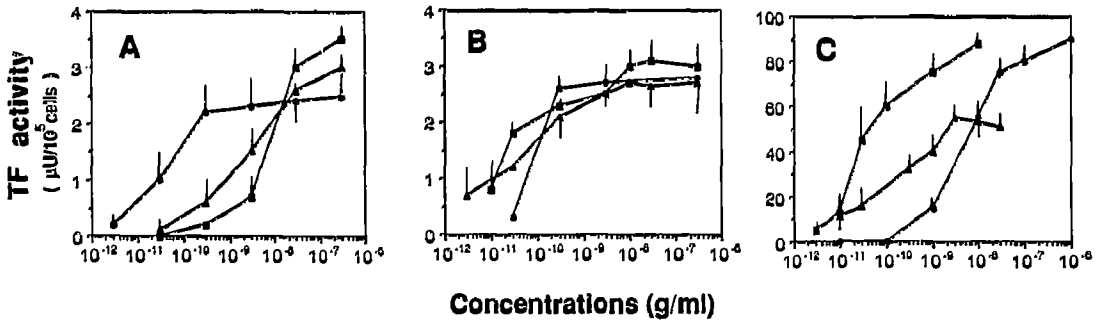

Fig. 2. Effect of LPS, IL-1β and TNF-α on TF expression in ABAE, HUVEC and monocytes. ABAE (A), HUVEC (B) and human monocytes (C) were incubated with increasing concentrations of LPS (●), IL-1β (■) or TNF-α (▲). Results are expressed as mean ± S.D. (n = 6).

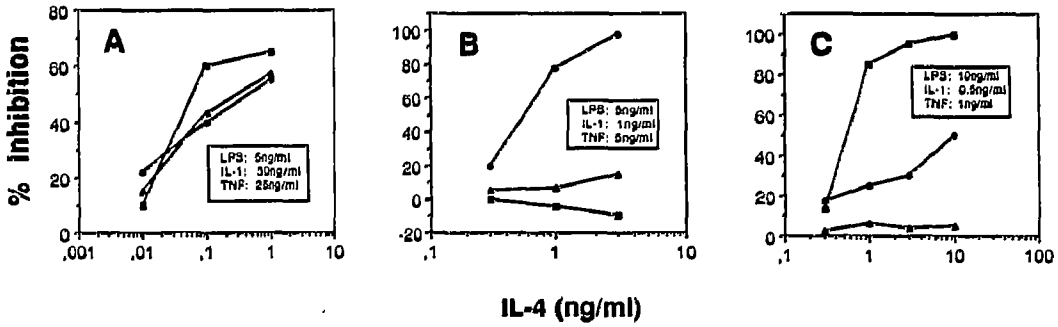

Fig. 3. Effect of IL-4 on LPS, IL-1β or TNFα-induced TF expression in ABAE, HUVEC and monocytes. Increasing concentrations of IL-4 were incubated with ABAE (A), HUVEC (B) or human monocytes (C) in the presence of PBS (○), LPS (●), IL-1β (■) or TNF-α (▲) at the indicated concentrations. Results are expressed as % inhibition of the control response (n = 6).

and therefore enable easier detection of an inhibitory effect of IL-4.

In human monocytes, IL-4 strongly reduced IL-1β-induced TF expression with an IC_{50} value of 0.4 ng/ml (Fig. 3C). With regard to the pyrogenic effect of TNF-α, IL-4 remained ineffective whereas it slightly inhibited the procoagulant effect of LPS with an IC_{50} value of 10 ng/ml.

Although pyrogen-induced pro-hemostatic changes in the endothelial cell surface have been known for several years, a substance that effectively counteracts these effects has so far not been described. Recently, IL-4, a product of activated T-cells, was shown to exert anti-inflammatory effects on human endothelial cells and monocytes [13–15] and to neutralize the pyrogen-induced down-regulation of thrombomodulin activity [6]. Our results together with the findings of others [6,13–15] provide evidence which shows that IL-4 effectively counteracts the procoagulant process resulting from the interaction of inflammatory mediators with endothelial cells and monocytes.

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