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IL-4 and IL-13 exhibit comparable abilities to reduce pyrogen-induced expression of procoagulant activity in endothelial cells and monocytes

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Endotoxin (LPS), interleukin-1β (IL-1) and tumor necrosis factor-α (TNF) increased the expression of tissue factor, a membrane-anchored glycoprotein that initiates blood coagulation on the surface of cultured bovine aortic endothelial cells (ABAE) and human monocytes. These compounds simultaneously reduced the amount of thrombomodulin on the endothelial cell surface, further contributing to the procoagulant activity of the endothelium or monocytes. On endothelial cells and monocytes, interleukin-4 (IL-4) and interleukin-13 (IL-13), a newly described lymphokine, both strongly inhibited LPS-induced tissue factor expression, a similar activity also being obtained with regard to the pyrogenic effects of IL-1 or TNF. When measured in parallel, IL-4 and IL-13 counteracted thrombomodulin down-regulation induced by LPS, IL-1 or TNF in endothelial cells. These results therefore show that both IL-4 and IL-13 protect the endothelial and the monocyte surface against inflammatory mediator- induced procoagulant changes.

Tissue factor; Thrombomodulin; Cytokines; IL-4; IL-13; Endothelial cell; Monocyte

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 antithrombotic 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 or TF expression in cultured vascular endothelial cells [6,7] and monocytes.

This work was aimed at determining the inhibitory effect of IL-13, a newly-described lymphokine with IL-4-like properties [8] on the expression of TF and TM on the surface of endothelial cells and monocytes induced by various inflammatory mediators.

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2. MATERIALS AND METHODS

2.1. *Cells*

Adult bovine aortic endothelial cells (ABAE) (passage 7–10) were isolated and cultured as already described [9] 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). Mononuclear cells were obtained from human heparinized blood as described by Boyum [10]. Cells were plated for 30 min at 37°C into 96-well microplates (10^5 cells/well). Non-adherent cells were then removed 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. [11]. Briefly, adherent cells were incubated for 18 h 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) or IL-13 which was cloned, produced and purified as described [8]. The medium was removed and the 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 μUnits of TF/10^5 cells.

2.3. *Determination of thrombomodulin activity*

Confluent endothelial cells were incubated with IL-4 or IL-13 in the

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presence of LPS (1 ng/ml), IL-1β (10 ng/ml) or TNF-α (1 ng/ml). After 24 h, cells were washed once with M 199. Purified human protein C (PC) (CTS, France) (60 mM) and purified human a-thrombin (CTS, France) (1 NIH U/ml) were incubated in 140 μl of M 199 containing 0.3% human serum albumin for 60 minutes at 37°C. Free thrombin was neutralized by an excess of recombinant hirudin (Sanofi Recherche, France) (20 ATU/ml) and the PC activity was determined by adding 100 μl of 0.2 mM S 2366. After 30 min, OD_{405} nm was measured.

3. RESULTS AND DISCUSSION

3.1. *Effect of LPS, IL-1β and TNF-α on TF and TM expression*

Unstimulated cells were devoid of TF activity, i.e. demonstrated the same insignificant hydrolysis of S2222 in the test but the addition of LPS, IL-1β or TNF-α to adherent endothelial cells and monocytes resulted in a dose-dependent expression of TF on the cell surface (Fig. 1A,B). The various inflammatory mediators showed different dose-responses for TF induction on the two cell types. LPS was the most potent stimulator on ABAE whereas IL-1β was more potent on monocytes. These compounds also induced the down-regulation of TM expression at the surface of the endothelial cells (Fig. 1C). These results confirm previous observations [5–7,12–14] and show that, on the surface of cultured endothelial cells, a procoagulant entity (TF) or an anti-coagulant one (TM) is simultaneously regulated by stimuli such as IL-1β or TNF-α which are susceptible to be secreted in pathological situations leading to thrombosis.

3.2. *Effect of IL-4 and IL-13 on pyrogen-induced TF and TM expression*

As shown in Fig. 2A, IL-4 and IL-13 counteracted in a dose-dependent manner IL-1β-, TNFα- and LPS-induced TF induction in ABAE. The IC50 values (concentrations which inhibited 50% of the pyrogen-induced

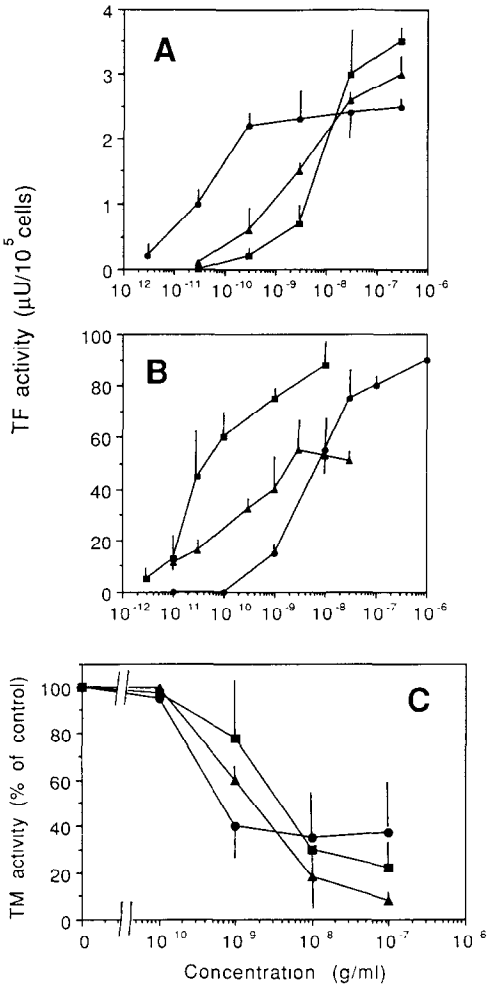

Fig. 1. Effect of LPS, IL-1β and TNF-α on TF and TM expression. ABAE (A,C) and human monocytes (B) were incubated with increasing concentrations of LPS (●), IL-1β (■) or TNF-α (▲). TF (A,B) and TM (C) expression was quantified as described in Section 2. Results are expressed as mean ± S.D. (n = 6).

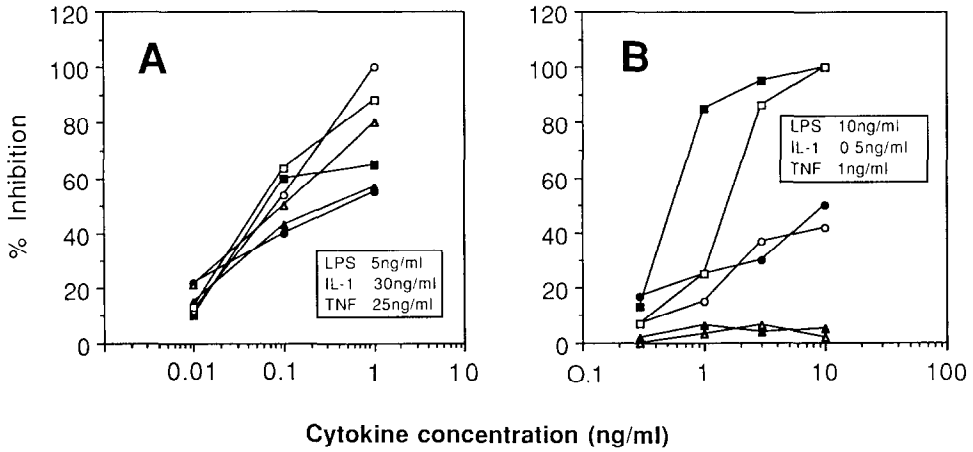

Fig. 2. Effect of IL-4 and IL-13 on LPS, IL-1β or TNFα-induced expression of TF in ABAE and monocytes. Increasing concentrations of IL-4 (full symbols) and IL-13 (empty symbols) were incubated with ABAE (A) or human monocytes (B) in the presence of LPS (●,○), IL-1β (■,□) or TNF-α (▲,△) at the indicated concentrations. TF expression was determined as described in Section 2. Results are expressed as % inhibition of the control response (n = 6).

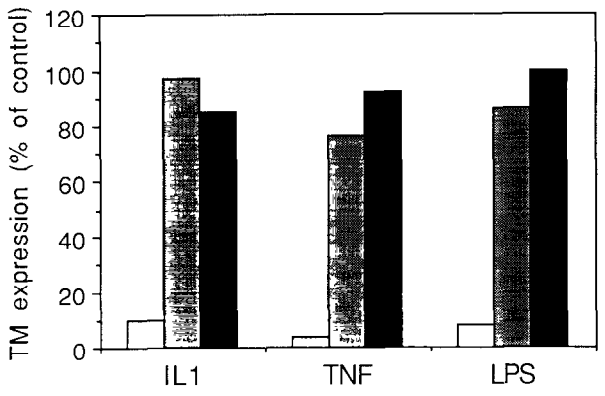

Fig. 3. Effect of IL-4 and IL-13 on pyrogen-induced TM down-regulation in ABAE. Confluent ABAE were incubated with LPS (1 ng/ml), IL-1β (10 ng/ml) or TNF-α (1 ng/ml) in the presence of the vehicle (empty bars), IL-4 (shaded bars) or IL-13 (full bars) (10 ng/ml). TM expression was determined as described under Section 2 and expressed as a percent of the control response observed in the absence of pyrogen ($n = 6$).

TF expression) with regard to the effect of all inflammatory mediators were between 0.05 and 0.5 ng/ml. In human monocytes (Fig. 2B), IL-4 and IL-13 strongly reduced IL-1β-induced TF expression with IC50 values of 0.4 and 1.3 ng/ml, respectively. With regard to the procoagulant effect of TNF-α, both IL-4 and IL-13 remained ineffective whereas they slightly inhibited the procoagulant effect of LPS.

In experiments performed in parallel, IL-4 and IL-13 reduced LPS-, IL-1β- or TNF-α-induced TM down-regulation in ABAE (Fig. 3). Such observations have already been described for IL-4 [6]. The concentrations of IL-4 and IL-13 (10 ng/ml) which counteracted LPS-, IL-1β- or TNF-α-induced TM down-regulation were in the same range as those shown to alter pyrogen-induced TF expression.

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 not so far been described. Recently, IL-4, a product of activated T-cells, was shown to exert anti-inflammatory effects on human endothelial cells and monocytes [15–17] and to neutralize the pyrogen-induced down-regulation of thrombomodulin activity [6]. We further provided evidence that IL-4 effectively counteracted the procoagulant process resulting from the interaction of inflammatory mediators with endothelial cells and monocytes [7].

We now show that IL-13, exhibits a comparable ability to reduce pyrogen-induced procoagulant activity in endothelial cells and monocytes but further work is now needed to determine if IL-13 is able to modulate the prothrombotic and proinflammatory properties of such compounds. The similar action of IL-13 and IL-4 on the procoagulant activity of monocytes and endothelial cells resembles their common inhibition of inflammatory cytokine synthesis by monocytes [8]. The activity of IL-13 on endothelial cells therefore adds another cell type to the wide range of target cells identified for this novel pleiotypic lymphokine.

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