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Interleukin-10 inhibits endotoxin-induced tissue factor mRNA production by human monocytes

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In Gram-negative septic shock, human monocytes synthesize and express on their cytoplasmic membrane tissue factor (TF), a potent activator of the coagulation cascades. The role of TF in triggering disseminated intravascular coagulation (DIC) in these patients appears to be clear. We report the suppressive effect of interleukin-10 (IL-10) on endotoxin-induced TF activity and antigen levels, and on the expression of TF mRNA levels in human monocytes. These results emphasize the potential therapeutic value of this cytokine in septic shock, a condition still associated with a high mortality rate.

Tissue factor; Interleukin-10; Monocyte; Endotoxin

1. INTRODUCTION

After incubation with Gram-negative endotoxin in vitro, blood monocytes synthesize and express membrane tissue factor (TF), a potent initiator of the coagulation cascades [1]. Mononuclear cells from patients with meningococcemia also show TF activity, that correlates with the prognosis of the disease [2]. Since anti-TF antibodies prevent or attenuate endotoxin- and TF-induced disseminated intravascular coagulation (DIC) in animal experimental models [3,4], the role of TF in triggering DIC in Gram-negative septic shock appears clear.

Interleukin-10 (IL-10) is a 35–40 kDa protein produced in various conditions of immune inactivation, by the T_H0 and T_H2 subsets of helper T cells, as well as by monocytes, macrophages and B cells [5]. It exerts a wide array of immunostimulatory effects on B cells [5] together with a number of immunosuppressive effects on monocytes and macrophages. In vitro, IL-10 inhibits monocyte production of several inflammatory mediators, including interleukin-1 (IL-1) α and β, interleukin-6, interleukin-8, granulocyte colony-stimulating factor and tumor necrosis factor (TNF) α, while it upregulates the expression of the IL-1 receptor antagonist [5]. These properties suggest that IL-10 might be a potent anti-inflammatory reagent in a variety of disease settings including septic shock.

The aim of this study was to investigate the effect of IL-10 on TF activity, immunological expression and mRNA in LPS-activated monocytes.

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2. MATERIALS AND METHODS

Mononuclear cell suspensions containing 20–30% monocytes and 70–80% lymphocytes were isolated by Ficoll-Hypaque gradient sedimentation as described in [6]. To obtain monocyte-enriched preparations (75–95%), monocytes were further purified by adherence to gelatin/fibronectin-coated petri dishes [7]. Cell suspension or adherent monocytes were incubated with or without IL-10 for 30 min at 37°C, under 5% CO_2, in RPMI medium supplemented as described in [6], before stimulation with 10 ng/ml LPS for 1–6 h. Purified human IL-10 was generously provided by DNAX, Palo Alto, CA. After incubation, the supernatant was discarded and cells were washed and stored for less than one week at −80°C until TF measurement or RNA extraction. Since monocytes are the only circulating cells to produce TF, mononuclear cell suspensions were used to measure TF activity and TF antigen levels, while adherent monocytes were used for RNA extraction; TF activity was also measured in the latter.

After thawing, cells were lysed and TF activity was assayed as described in [6,7]. PCA was expressed as TF milliunits/10^6 monocytes. To determine what proportion of PCA was TF-initiated, residual PCA was measured, in some experiments, after incubating the cell lysate with a pool of neutralizing anti-TF monoclonal antibodies (TF8-5G9, TF8-6B4, and TF9-9C3) at 10 μg/ml. The antibodies were generously given by T.S. Edgington [8].

Total RNA was extracted by the guanidinium-thiocyanate/cesium chloride method [7]. Filters were hybridized for 24 h at 42°C, simultaneously to the TF and G3PDH cDNA probes. The autoradiograms were scanned by means of laser densitometry, using the Sebia Preference R apparatus, for the relative levels of mRNA in treated and untreated monocytes.

cDNA probes were labeled with [α-^{32}P]dCTP (3000 Ci/mmol) by random hexamer primer extension, using the Amersham Multiprime kit. The 641 bp TF cDNA probe kindly provided by T.S. Edgington [9] was excised from plasmid pUC8 with *Eco*RI. The G3PDH probe was purchased from Clontech (Palo Alto, CA).

TF antigen was determined by an ELISA method using the Imubind TF kit (American Diagnostica Inc., Greenwich) according to the manufacturer's instructions, with slight modifications. Cells were solubilized in PBS containing 1% Triton X-100 and directly transferred to the ELISA plate.

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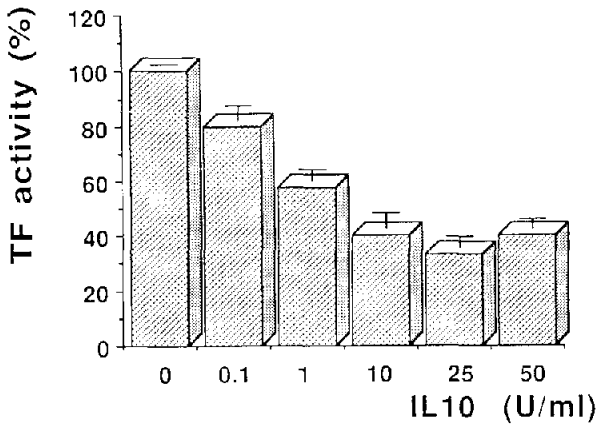

Fig. 1. IL-10 concentration-dependent inhibition of LPS-induced TF activity. Mononuclear cell suspensions were pretreated with increasing concentrations of IL-10 for 30 min and subsequently stimulated with LPS (10 ng/ml) for 4 h. Data are the percentage of the maximal response obtained in the absence of IL-10. Results are given as the mean ± S.E.M. of four experiments.

3. RESULTS AND DISCUSSION

We first investigated the effect of graded concentrations of IL-10 on LPS-induced functional TF activity in human monocytes. Freshly collected mononuclear cells had no detectable procoagulant activity (PCA) (clotting times >160 s). After incubation, the PCA of LPS-treated cells was 2732 ± 230.7 milliunits/10^6 monocytes (mean ± S.E.M. of 4 separate experiments). PCA was identified as TF, since more than 99% was inhibited by a mixture of neutralizing monoclonal antibodies to human TF. Increasing IL-10 concentrations (0.1–50 U/ml) inhibited LPS-induced TF activity of mononuclear cells in a concentration-dependent manner. IL-10 at 25 U/ml inhibited 70% of total TF activity (Fig. 1); no further inhibition was obtained at higher concentrations of IL-10.

In parallel to the effect on TF activity, IL-10 had a concentration-dependent inhibitory effect on TF antigen levels measured by means of an ELISA. As shown in Fig. 2, TF activity and antigen levels measured in paired experiments correlated strongly ($r = 0.927$, $P < 0.001$), ruling out the involvement of the TF pathway inhibitor (TFPI) in the observed effect [10].

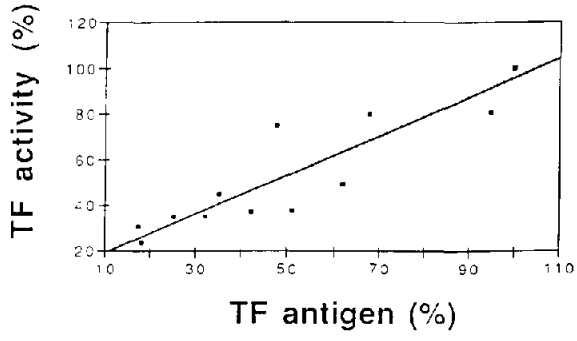

Fig. 2. Correlation between TF antigen expression (ELISA) and TF activity of LPS-exposed monocytes, in the presence of increasing concentrations of IL-10 (0, 0.1, 1, 10 and 25 U/ml). The results are given as the percentage of maximal TF expression induced by LPS (10 ng/ml) in the absence of IL-10.

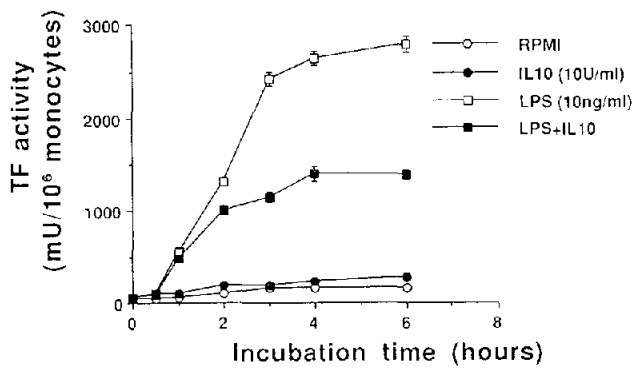

Fig. 3. Kinetics of IL-10 inhibition of LPS-induced TF activity from 0 to 6 hours (mean ± S.E.M., $n = 3$). Mononuclear cells were incubated for 30 min, 1, 2, 3, 4 and 6 h with RPMI medium alone, LPS, IL-10 or LPS + IL-10.

We then compared TF activity of monocytes exposed to LPS for various times (0–4 h), with or without IL-10 pretreatment (Fig. 3). After the addition of LPS, TF activity increased gradually from 0 to 6 h. When IL-10 was added, TF activity decreased as early as the second hour after the addition of LPS, the effect was maximum at 3 h and continued at 4 and 6 h. Thus IL-10 acted on an early step of TF production, as it does on IL-1 and TNF alpha production [11].

We then evaluated the effect of IL-10 on TF mRNA levels in LPS-stimulated adherent monocytes. TF mRNA was not detectable in unstimulated monocytes (Fig. 4). Exposure to 10 ng/ml of LPS for 4 h was associated with high TF mRNA levels. The addition of

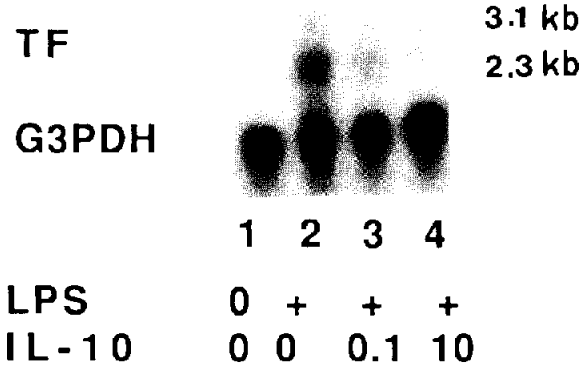

Fig. 4. IL-10 induced a concentration-dependent inhibition of LPS-induced TF mRNA. Five μg of total RNA was analysed by Northern blotting. RNA was extracted from untreated monocytes (1) and from cells exposed for 4 h to 10 ng/ml LPS (2) or to LPS + IL-10 at 0.1 (3) and 10 (4) U/ml. The membranes were hybridized to TF and G3PDH probes.

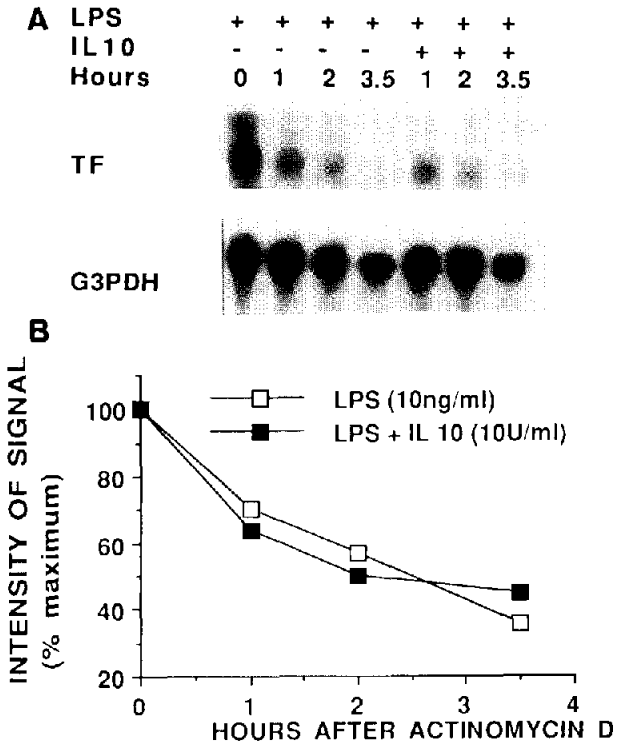

Fig. 5. Effect of IL-10 on the stability of LPS-induced TF mRNA. Monocytes were exposed to LPS for 2 h to induce TF mRNA. Actinomycin D (10 μg/ml) was added, followed by IL-10 (10 U/ml). Total RNA was harvested at the indicated times after the addition of actinomycin D, and analyzed by Northern blotting (A). Autoradiograms were scanned by means of laser densitometry. The stability of the 2.3 kb TF mRNA species is shown (B).

0.1 and 10 U/ml IL-10 was associated with a concentration-dependent decrease in TF mRNA (Fig. 4). We checked that IL-10 had the same inhibitory effect on TF activity generated by adherent monocytes and cells in suspension (data not shown).

Activation of monocytes and macrophages plays an important role in immune responses against microorganisms and tumors. IL-10 has already been shown to inhibit the secretion of a number of cytokines by inhibiting cytokine-specific mRNA synthesis induced by LPS [11]. In this study, we show that IL-10 also inhibits TF production at the mRNA level.

The rate of mRNA degradation is an important regulatory mechanism that may control the level of gene expression. The half-life of the TF mRNA was examined, with and without IL-10 pretreatment. As shown in Fig. 5, the yield of total RNA decreased similarly in the presence and absence of IL-10. The half-life of TF mRNA was approximately 2 h in both conditions. This indicates that IL-10 does not alter the half-life of TF mRNA and suggests an effect of IL-10 at the transcriptional level. Conflicting results have been reported as concerns the mechanism of the inhibitory effect of IL-10 on TNF-α mRNA expression. According to de Waal Malefyt et al. [12], the inhibition of TNF-α secretion, like that of other cytokines, occurs at the transcriptional level, whereas Bogdan et al. [11] found no suppressive effect on the transcription of the corresponding genes and concluded that IL-10 promotes the degradation of cytokine mRNAs.

Two studies from independent laboratories recently showed that IL-10 was highly effective at protecting mice from lethal endotoxin shock, an undesirable monokine-mediated inflammatory reaction [13,14]. Interestingly, this was the case whether IL-10 was administered concurrently with or 30 min after the injection of endotoxin [14]. The protective effect was associated with a substantial decrease in endotoxin-induced TNF-α release.

The intravascular expression of TF, and the increase in plasma levels of TNF alpha and IL-1, have been implicated in the onset of the multiple organ failure syndrome observed in Gram-negative septic shock [15]. The demonstration that IL-10 is capable of inhibiting the production of both molecules simultaneously is thus of considerable interest for the treatment of this often fatal condition [16].

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