Interleukin-4 Stimulates Human Monocytes to Produce Tissue-Type Plasminogen Activator

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Tissue-type plasminogen activator (t-PA) is involved in the lysis of blood clots (fibrinolysis) and is used clinically for this purpose. Endothelial cells are one source of the t-PA present in blood. We report here that interleukin-4 (IL-4) (0.1 to 0.25 U/mL; 1 to 3 x 10^{-11} mol/L), but not interferon-γ (IFN-γ), elevates t-PA messenger (m)RNA expression and secretion of t-PA activity by human monocytes, with the maximum response at 2.5 U/mL. Supernatant t-PA activity was detected within three hours of exposure to IL-4 and maximum activity within six hours. Thus, IL-4 may control fibrin deposition at sites of inflammation during cell-mediated immune responses, as well as having a therapeutic role in thrombolysis.

I N THE LIVING ORGANISM, a dynamic equilibrium or hemostatic balance exists between fibrin clot formation and resolution. The fibrinolytic system comprises the proenzyme plasminogen, which can be converted to the active serine protease, plasmin, by plasminogen activators (PAs). The two types of PA, tissue-type PA (t-PA) and urokinase-type PA (u-PA), are serine proteases and are products of separate genes. Because of its affinity for fibrin, t-PA is likely to be the major PA involved in vascular hemostasis and vessel patency, as well as controlling fibrin turnover at sites of chronic inflammation. The administration of t-PA as a thrombolytic agent for the treatment of myocardial infarction is now well-established and its potential for treatment of pulmonary embolism and deep vein thrombosis is becoming apparent. However, the problems associated with t-PA therapy, namely bleeding and cost, prompt the search for improved thrombolytic regimens.

Until recently, endothelial cells were considered to be the source of circulating t-PA. We have reported that human monocytes can produce t-PA in vitro when stimulated by lipopolysaccharide (LPS) or by LPS together with interferon-γ (IFN-γ), suggesting that local activation of monocytes at sites of fibrin deposition may contribute to thrombolysis. Unfortunately, LPS and LPS with IFN-γ also stimulate monocytes to produce inflammatory mediators. We report here that interleukin-4 (IL-4), which does not induce inflammatory mediators, is a potent stimulus for t-PA production by human monocytes.

MATERIALS AND METHODS

Monocyte isolation and culture. Monocytes (≥95% enriched) were isolated from peripheral venous blood by countercurrent centrifugal elutriation and cultured (0.8 to 1.0 x 10^6 in 1 mL) under LPS-free conditions for 18 hours as previously described. Recombinant human IL-4 (≤400 U/μg where 1 U/mL stimulated half-maximal growth of phytohemagglutinin (PHA)-activated T cells; DNAX Research Institute, Palo Alto, CA) was added from 0 to 5.0 U/mL. When indicated, LPS from Escherichia coli 0111:B4, purified by the Westphal method (Difco, Detroit), was added at 100 ng/mL; recombinant human IFN-γ (Dr E. Hochuli, Hoffmann-La Roche, Basel, Switzerland) was added at 100 U/mL.

Assay of PA activity. PA activity was assayed by measuring the degradation products of fibrin, and expressed according to the activity of a t-PA standard (National Institute for Biological Standards and Control, London). More than 95% of the activity measured was plasminogen-dependent.

Characterization of PA activity. Before assaying, PA-containing fluids were incubated for one hour at 37°C with IgGs (1 μg/mL final concentration) isolated from rabbit antisera to human u-PA and t-PA (Dr W-D. Schleuning, Schering AG, Berlin) using Protein A sepharose CL-4B (Pharmacia, Uppsala, Sweden). The mouse myeloma IgG, HOPCY, was used as an irrelevant antibody. The u-PA standard was from Leo Pharmaceutical Products, Denmark; the t-PA standard was a culture supernatant from the MM138 melanoma cell line (Dr R. Whitehead, Ludwig Institute for Cancer Research, Melbourne). SDS-casein zymography was performed to further characterize PA activity. Clear bands of lysis appeared where PA had converted plasminogen to plasmin, and low molecular weight markers (Pharmacia) were used to define the main caseinolytic bands. Immunoprecipitation using the PA-specific IgGs also confirmed the identity of the standard PA bands.

Detection of messenger (m)RNA in human monocytes. Total monocyte RNA was prepared as previously described and fractionated (5 μg/lane) on a formaldehyde-containing 1% agarose gel before transferring to Genescreen Plus nylon membrane (Dupont, Boston). For t-PA, the filter was hybridized overnight at 60°C in a standard hybridization buffer containing 32P-labeled t-PA complementary (c)RNA (Dr W-D. Schleuning). After hybridization, the filter was washed and treated with 1 μg/mL RNaseA. For u-PA (cDNA, Dr F. Blasi, University of Copenhagen) and the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hybridization using cDNAs was essentially as previously described, except that the hybridizations were performed at 45°C.

RESULTS

IL-4 (2.5 U/mL, 3 x 10^{-10} mol/L) increased the PA activity of both untreated monocytes and monocytes treated with LPS or with LPS + IFN-γ (Fig 1A). When mean activities for triplicate cultures from ten donors were compared, IL-4 (2.5 U/mL) stimulated PA activity in control monocytes from nondetectable levels to 0.16 ± 0.03 IU/10^6 cells (mean ± SEM). For nine monocyte donors, the mean PA activity induced by LPS was increased by IL-4 from 0.06 ± 0.02 to 0.23 ± 0.07 IU/10^6 cells, while the mean PA activity induced by LPS with IFN-γ was increased from...
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Fig 1. PA activities in the supernatants of IL-4-stimulated human monocytes. (A) Levels of PA activity. Monocytes from a representative donor were incubated with 2.5 U IL-4/mL, alone (C), or with LPS (100 ng/mL) or LPS (100 ng/mL) + IFN-γ (100 U/mL). The mean PA activity in the supernatants from triplicate cultures ± SEM, expressed according to the number of cells plated, is shown; there was no significant change in the number of monocytes in any treatment group after 18 hours in culture. ND, not detected. (B) Immunologic characterization. PA standards (0.1 IU/mL) and pooled culture supernatants from duplicate 18-hour cultures treated with 2.5 U IL-4/mL were incubated with PA-specific IgGs (see Materials and Methods). PA levels are expressed as 125I-fibrinolytic activity (cpm). ND, not detected. (C) Characterization by SDS-casein zymography. Pooled supernatants from duplicate cultures were obtained from: lane 1, cell-free cultures (showing weak caseinolytic bands due to 1% fetal calf serum); lane 2, control monocytes; lane 3, monocytes incubated with IL-4; lanes 4 and 5, monocytes incubated with LPS without or with IL-4, respectively; lanes 6 and 7, monocytes incubated with LPS and IFN-γ without or with IL-4, respectively; lane 8, t-PA standard; lane 9, u-PA standard. A 36-Kd breakdown product was detected only for the u-PA standard. Weak caseinolytic bands due to high molecular weight serum components (> 85 Kd) were seen for all supernatants.

0.27 ± 0.04 to 0.64 ± 0.12 IU/10⁶ cells. Deliberate addition of lymphocytes to the highly purified monocyte cultures did not increase PA activity, suggesting that the PA detected was indeed a monocyte product.

Since the PA activity detected in the culture supernatants of IL-4-stimulated monocytes was blocked by anti-t-PA IgG but not by anti-u-PA IgG (Fig 1B), this activity, like the LPS (+ IFN-γ)-induced PA activity, was of the t-PA type. Using SDS-casein zymography that allows PA detection in the presence of inhibitors, bands of 52 Kd and 70 Kd corresponding to u-PA and t-PA standards, respectively, were measured in the supernatants of monocytes incubated with IL-4 (Lane 3, Fig 1C). Human monocytes can secrete u-PA; however, the lack of u-PA activity in culture supernatants probably reflects the secretion by monocytes of a large excess of PA inhibitor type-2 that preferentially inhibits u-PA. Gels corresponding to the activities in Fig 1A are shown in Fig 1C; the relative intensities of the 70 Kd t-PA lytic bands closely follow the PA activities measured in the supernatants, consistent with the absence of significant t-PA inhibitors.

The t-PA activity was first detected in monocyte culture...
supernatants after three hours exposure to 2.5 U IL-4/mL. (Fig 2) and maximal t-PA expression was measured at six hours (Fig 2). In contrast, the t-PA activity induced by LPS with IFN-γ was still increasing after 18 hours (Fig 2). Cycloheximide (5 μg/mL) blocked the IL-4-stimulated t-PA activity indicating a requirement for protein synthesis (data not shown). Significant t-PA production was detected in response to 0.1 to 0.25 U IL-4/mL with a maximum response at 2.5 U IL-4/mL (data not shown).

IL-4–induced t-PA activity was inhibited by actinomycin D (2 μg/mL), suggesting enhanced t-PA gene expression. This conclusion is supported by Northern analysis, which showed that IL-4 increased the t-PA mRNA level (Lane 2, Fig 3). Unlike LPS (Lane 3, Fig 3), IL-4 did not stimulate expression of u-PA mRNA.

The PA activities in monocyte lysates were predominantly u-PA, considerably lower than those in culture supernatants, and not significantly altered by LPS ± IFN-γ. IL-4 stimulated a significant increase in cell-associated PA activities; for monocytes from seven donors incubated with 2.5 U IL-4/mL for 18 hours, the mean cell-associated PA activity ± SEM increased from 0.0012 ± 0.0005 to 0.0062 ± 0.0020 IU/10⁴ cells. In support of the Northern blots (Fig 3), this increase was shown to be t-PA activity by SDS-casein zymography (data not shown).

We have previously reported that IL-4 mimics anti-inflammatory glucocorticoids by inhibiting monocyte production of tumor necrosis factor α (TNFα), IL-1, and prostaglandin E₂ (PGE₂). Hence, it was proposed that IL-4 might have a role as an anti-inflammatory and immunosuppressive agent. For a representative donor, dexamethasone (10⁻⁷ mol/L) inhibited the t-PA activity (mean ± SEM, n = 3) induced by IL-4 (2.5 U/mL) from 0.31 ± 0.02 to 0.06 ± 0.01 IU/10⁶ cells. In the presence of LPS ± IFN-γ, IL-4 enhanced (Fig 1) and dexamethasone suppressed t-PA activities (data not shown). Thus, the similarity in action between IL-4 and glucocorticoids on human monocytes is not a general phenomenon.

DISCUSSION

We have shown that the lymphokine IL-4 (0.1 to 0.25 U/mL) stimulates human monocytes to produce t-PA, as identified by biochemic and immunologic criteria. Northern analysis and inhibition by actinomycin D suggest that t-PA production is regulated at the level of gene transcription.

We have previously noted that IL-4 and IFN-γ have opposite effects on the production of TNFα and IL-1 by
LPS-stimulated human monocytes, the IL-4 having an inhibitory action. Similarly, IL-4 and IFN-γ have opposite actions on B-cell functions. However, both IL-4 and IFN-γ potentiated the action of LPS for t-PA production (Fig 1). The mechanism of LPS + IFN-γ for t-PA induction may differ from that of IL-4, as implied by the kinetics data (Fig 2) and by the differential induction of cell-associated t-PA activity by IL-4 and LPS + IFN-γ.

There is evidence that fibrin, formed as a result of lymphokine activation of monocyte/macrophage procoagulant activity, may play a role in immune reactions associated with diseases such as rheumatoid arthritis, glomerular nephritis, and granulomatous disease. Since monocytes activated by IL-4 should remove fibrin as a result of t-PA activity, we suggest that the amount of fibrin at sites of inflammation and in T-lymphocyte-infiltrated atherosclerotic plaques may reflect an uneven balance in activities between two functionally opposing lymphokines, IL-4 and macrophage procoagulant-inducing factor. IL-4 may be able to modulate the t-PA activity of monocyte-derived foam cells in atherosclerotic plaques and therefore influence the resolution of fibrin in early lesions.

The equilibrium between fibrinolysis and hemostasis is believed to involve a significant cellular-phase component under both normal and physiologically or pharmacologically altered conditions. We have suggested a role for monocytes through their ability to express t-PA activity. Furthermore, IL-4 may prove a useful agent to stimulate local t-PA activity, thereby providing an alternative approach to thrombus resolution and the treatment of myocardial infarction.

Acknowledgment

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References

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