

Epinephrine Promotes IL-8 Production in Human Leukocytes via an Effect on Platelets

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Summary

Interleukin-8 (IL-8) is generally accepted to be an important mediator of a number of acute and chronic inflammatory diseases and is produced by monocytes upon stimulation by lipopolysaccharide (LPS). Epinephrine has been reported by several groups to suppress activation of monocytes in response to LPS, and the aim of the present study was to examine the effect of epinephrine on LPS induced IL-8 production using whole blood as a model system. Epinephrine increased LPS induced IL-8 production in a dose-dependent manner in the whole concentration range (0.001–100 μM) and 1 μM epinephrine increased IL-8 levels with 125%. Epinephrine *per se* had no effect on IL-8 levels. The potentiating effect of epinephrine was mediated by blood platelets, since IL-8 levels in samples containing platelets and stimulated with LPS and epinephrine (1–100 μM) were significantly higher ($p < 0.05$) than in control samples containing no platelets. This effect of platelets seemed to be due to platelet release products, since addition of 25 μl platelet lysate supernatant to whole blood increased LPS induced IL-8 production with 100% and a similar effect was observed in freshly isolated mononuclear cells resuspended in plasma. Upon addition of 50 $\mu\text{g/ml}$ of the carboxyterminal peptide of platelet factor 4 (PF4(58-70)) to whole blood, LPS stimulated IL-8 levels were increased with 115%, whereas in mononuclear cells, 20 $\mu\text{g/ml}$ PF4(58-70) enhanced IL-8 production with 40%. We demonstrate for the first time that epinephrine promotes LPS induced production of IL-8 in whole blood via an effect on blood platelets. This potentiating effect of platelets is shown to be due to platelet granule contents, and platelet factor 4 (PF4) is suggested to be one of several platelet granule proteins promoting LPS induced IL-8 production in whole blood.

Introduction

The CXC-chemokine interleukin-8 (IL-8) was originally detected in the culture supernatant of LPS stimulated human blood monocytes (1, 2). IL-8 is now known to be produced not only by monocytes, but also by macrophages, lymphocytes, granulocytes, endothelial cells, and

by a variety of tissue and tumor cells in response to inflammatory stimuli such as LPS, viruses, lectins, IL-1, and TNF α . IL-8 is closely associated with acute inflammatory conditions like sepsis syndrome, adult respiratory distress syndrome (ARDS) and ischaemia-reperfusion injury. It is generally agreed upon that IL-8 plays an essential role in neutrophil recruitment to and activation at the inflammatory site (reviewed in 3, 4).

The catecholamine and stress hormone epinephrine has been found by several groups to attenuate cytokine expression induced by LPS. According to Sekut et al. (5), epinephrine reduced LPS stimulated TNF α production in THP-1 cells by interacting with β -adrenoceptors and thereby causing an elevation in intracellular cAMP concentration. Since TNF α mRNA levels remained unchanged, the negative effect of epinephrine was thought to be due to inhibition of translation (6). Epinephrine inhibited LPS induced TNF α secretion in a dose-dependent way and increased IL-10 production in whole blood *ex vivo*, and intravenous injection of epinephrine in volunteers 3 h before infusion of LPS resulted in a decrease in TNF α levels *in vivo* (7). These reports are in accordance with our own observations that epinephrine inhibits LPS induced monocyte-associated TF expression in whole blood (Østerud, unpublished).

We wished therefore to study the effect of epinephrine on LPS induced IL-8 secretion in human blood. Whole blood or blood cells resuspended in autologous plasma constitute excellent *ex vivo* models for studies of cytokine release and coagulation activation, since blood cells are kept in their natural environment. Furthermore, in whole blood cellular interactions are largely preserved and priming effects due to contamination and handling of cells under isolation are kept at a minimum (8). Avoiding potential artefacts due to the effect of adherence on cell function (9–13) was especially important in the present study, since IL-8 and MCP-1 genes have been reported to be induced in monocytes by adherence to plastic surfaces (14). In this paper we report that epinephrine increased LPS induced IL-8 secretion in human whole blood in a platelet-dependent manner. The increasing effect of platelets was found to be due to platelet contents, and platelet factor 4 (PF4) is suggested to be one of several platelet granule proteins promoting LPS induced IL-8 production in whole blood.

Materials and Methods

Reagents

The anticoagulants heparin and hirudin were obtained from Novo Nordisk, Copenhagen, Denmark and Hoechst, Marburg, Germany, respectively. LPS (*E. coli* 026:B6) was purchased from Difco Laboratories (Detroit, Michigan, USA), and PMA and TNF α were from Sigma Chemical Company, St. Louis, MO, USA. Epinephrine, Lymphoprep and Polymorphprep were from Nycomed, Oslo, Norway.

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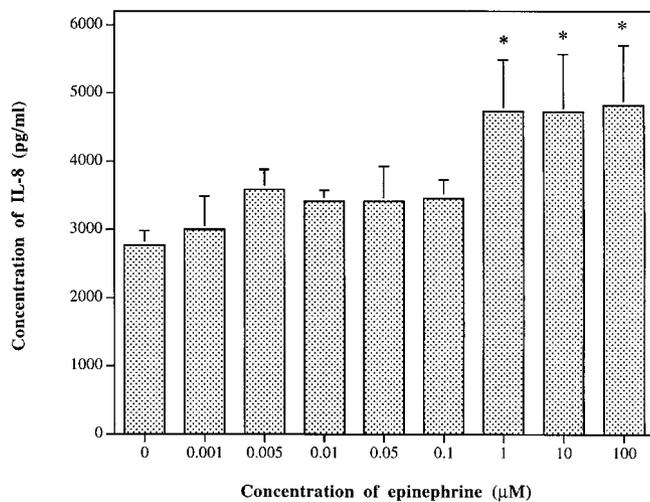


Fig. 1 The effect of epinephrine on LPS induced IL-8 production in whole blood is concentration-dependent. 1 ml samples of whole blood samples anticoagulated with hirudin were incubated with LPS (5 ng/ml) and saline (control) or increasing concentrations of epinephrine (0.001, 0.005, 0.01, 0.05, 0.1, 1, 10, and 100 µg/ml) for 2 h at 37° C and 160 rpm. At the end of the incubation time 100 µl 2% EDTA was added and PPP was isolated and frozen at -70° C. IL-8 was quantified using an ELISA Biotrak kit. Results are expressed as means with SEM, n = 4. Values were considered significantly different in the case of $p < 0.05$ by the Newman-Keuls test of post hoc comparison of means (* = $p < 0.05$)

Preparation of Platelet Lysate Supernatant

Blood was drawn into a plastic syringe containing 1.5% Na₂EDTA to give a final concentration of 0.15% Na₂EDTA. After centrifugation at 180 g for 15 min, platelet rich plasma (PRP) was removed and platelet and leukocyte numbers were counted on a Sysmex K1000 cell counter (Toa Medical Electronics Co., Kobe, Japan). PRP contained on the average 0.03×10^6 leukocytes/ml. Platelets were washed twice with citrate-saline buffer (0.9% NaCl, 0.32% trisodium citrate) by centrifugation at 1950 g and 4° C for 15 min, and finally resuspended in citrate-saline buffer to a concentration of 3×10^9 platelets/ml. After freezing and thawing the platelet solution five times, platelet debris was removed by centrifugation at 12,400 g and 4° C for 15 min. The supernatant was stored at -70° C.

Peptide Synthesis

A carboxy-terminal tridecapeptide of human platelet factor 4 (PF4(58-70)) with the sequence H₂N-PLYKIIKKLLES-COOH was synthesized as described previously (15).

Blood Sampling

Human blood was drawn into a plastic syringe with a 19-gauge needle and distributed into polycarbonate tubes containing heparin or hirudin at final concentrations of 10 U/ml and 10 µg/ml, respectively.

Isolation of Cells

Anticoagulated whole blood was centrifuged at 180 g for 15 min, PRP was removed and substituted with autologous plasma. Mononuclear cells and granulocytes were isolated on a Lymphoprep/Polymorphprep gradient by centrifugation at 530 g for 20 min and washed in sterile saline followed by centrifugation at 1450 g for 10 min. Following hypotonic lysis of contaminating red blood cells, the leukocytes were resuspended in PPP (platelet poor plasma).

Test System

Anticoagulated whole blood samples or cell samples were incubated with LPS and various additives in a shaker incubator at 37° C and 160 rpm for different time intervals. The reaction was stopped by adding 100 µl 2% Na₂EDTA/ml whole blood or cells and the samples were centrifuged at 1450 g for 10 min. PPP was removed and stored at -70° until testing.

Quantification of IL-8 and MCP-1

IL-8 in PPP samples was analysed using an ELISA Biotrak kit (Amersham, Buckinghamshire, England), and MCP-1 was measured using a Quantikine ELISA kit from R & D systems, Minneapolis, USA.

Statistics

All statistical analyses were performed by Statistica 3.0b (Statsoft Inc., Tulsa, OK, USA). Data were analyzed by 1- or 2-way analysis of variance (ANOVA) followed by Scheffe F-test or Students paired t-test, as appropriate. P-values < 0.05 were considered significant. All results are reported as means ± SEM.

Results

The Effect of Epinephrine on LPS Induced IL 8 Production in Whole Blood

Since epinephrine has been reported to attenuate TNFα production in whole blood in response to LPS (6, 7), we were interested in examining if epinephrine at concentrations commonly used to activate platelets in whole blood (16, 17) could have any effect on LPS induced IL-8 generation. Interestingly, epinephrine increased LPS induced IL-8 production dose-dependently within the range 0.001 µM to 1 µM (global

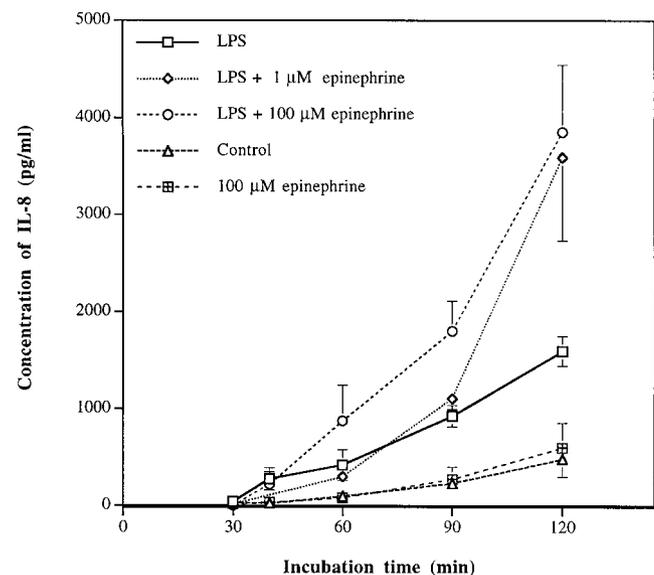


Fig. 2 The effect of epinephrine and LPS on IL-8 production in whole blood. 1 ml samples of whole blood anticoagulated with hirudin were incubated with saline (control), epinephrine (100 µM), LPS (5 ng/ml), and LPS (5 ng/ml) and epinephrine (1 and 100 µM) for 30, 60, 90 and 120 min at 37° C and 160 rpm. At the end of the incubation time 100 µl 2% EDTA was added and PPP was isolated and frozen at -70° C. IL-8 was quantified using an ELISA Biotrak kit. Results are expressed as means with SEM, n = 3. Values were considered significantly different in the case of $p < 0.05$ by the Scheffe F-test of post hoc comparison of means

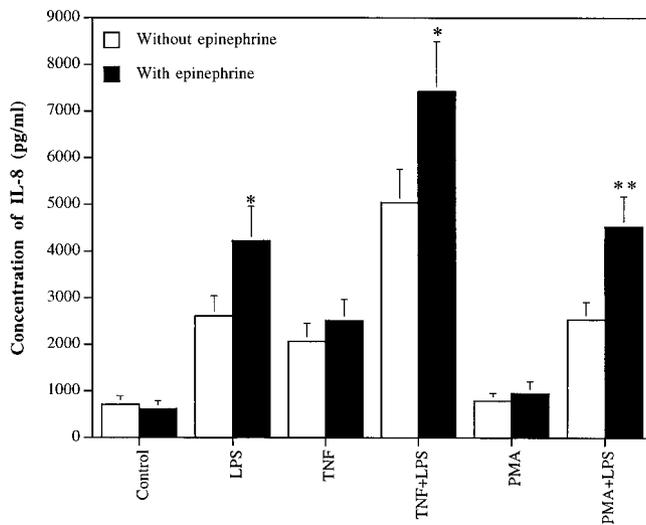


Fig. 3 The effect of epinephrine on IL-8 levels in whole blood stimulated with LPS, TNF α or PMA. 1 ml whole blood samples anticoagulated with hirudin were stimulated with saline (control), LPS (5 ng/ml), TNF α (10 ng/ml), PMA (5 ng/ml) or epinephrine (100 μ M) at 37 $^{\circ}$ C in a shaker incubator at 160 rpm. After 2 h incubation 100 μ l 2% EDTA was added, and PPP was isolated and frozen at -70 $^{\circ}$ C. IL-8 was quantified using an ELISA Biotrak kit. Data are presented as means with SEM, n = 6. Values were considered significantly different in the case of $p < 0.05$ by paired Students t-test (* = $p < 0.005$ compared to the same sample without adrenaline; ** = $p < 0.001$ compared to the same sample without adrenaline)

p -value = 0.001), the effect being significant ($p < 0.01$) when epinephrine concentrations of 1, 10, or 100 μ M were used (Fig. 1). Fig. 2 shows that addition of 100 μ M epinephrine *per se* had no effect on IL-8 levels in hirudinized blood as compared to control samples. 100 μ M epinephrine had an enhancing effect on LPS induced IL-8 production already at 60 min of incubation, whereas 1 μ M epinephrine did not have any enhancing effect before 120 min. At 120 min of incubation either epinephrine concentrations used (1 μ M and 100 μ M) potentiated the effect of endotoxin on IL-8 generation with 130%.

In another series of experiments (Fig. 3), epinephrine was also shown to amplify the generation of IL-8 in blood costimulated with LPS and TNF α by 50% ($p < 0.005$) and in blood costimulated with LPS and PMA by 84% ($p < 0.001$), respectively. The effect of epinephrine/TNF α and epinephrine/PMA on LPS induced IL-8 production was only additive and not synergistic. Although TNF α *per se* also induced substantial IL-8 production, epinephrine had no enhancing effect on TNF α induced IL-8 production.

The Potentiating Effect of Epinephrine on LPS Induced IL-8 Production is Platelet-dependent

To see whether the effect of epinephrine on IL-8 production in whole blood was mediated by its platelet activating properties (reviewed in 18), whole blood was centrifuged to remove platelet rich plasma. The remaining blood cells were then recombined with either platelet poor plasma (PPP) or platelet rich plasma (PRP), and subsequently incubated with LPS in the presence or absence of epinephrine. Fig. 4 shows that the effect of epinephrine is dependent on the presence of blood platelets. LPS induced IL-8 production was increased strongly and significantly ($p < 0.05$) in samples stimulated with epinephrine

(1, 10, 100 μ M) and containing platelets, compared to samples stimulated with LPS and epinephrine but without platelets present. In the absence of epinephrine, addition of platelets had no influence on IL-8 levels as compared to samples without platelets.

The Effect of Platelet Secretion Products on LPS Induced IL-8 Secretion

Next we wished to examine if the platelet effect was dependent on intact platelets or if it could be mimicked by isolated platelet contents. To this end, whole blood was incubated with 5 ng/ml LPS and increasing concentrations of platelet lysate supernatant. As shown in Fig. 5, platelet lysate supernatant increased LPS induced IL-8 production in whole blood dose-dependently (global p -value = 0.0003). Addition of 5 or 10 μ l/ml supernatant tended to increase IL-8 secretion with 50%. Addition of 25 and 50 μ l/ml supernatant produced strong and significant effects on IL-8 production: an increase of 100 and 140%, respectively, was measured. In the subsequent experiment we examined whether platelet lysate supernatant could have a direct effect on mononuclear cells. As illustrated in Fig. 6, platelet lysate supernatant increased IL-8 secretion dose-dependently also in isolated mononuclear cells suspended in PPP (global p -value = 0.003). By the Scheffe F-test, 15, 25 and 50 μ l/ml supernatant had a significant and increasing effect on IL-8 production ($p < 0.05$). Individual differences in the response to platelet lysate supernatant were very large, and we chose to show the individual responses of three persons. Platelet lysate *per se* had no effect on IL-8 production in the absence of LPS and did not contain any detectable amounts of IL-8 (data not shown).

Since PMN are known to produce IL-8 in response to LPS (19, 20), and since the supernatant of collagen stimulated platelets has been shown to potentiate PMN reactivity to GM-CSF (21), it was of interest to examine whether platelet lysate supernatant could have any effect on LPS induced IL-8 production in PMN. PMN resuspended in PPP (3 \times

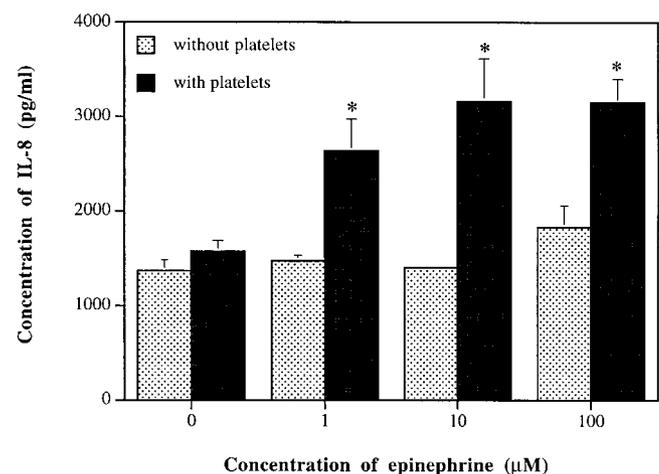


Fig. 4 The effect of epinephrine on LPS induced IL-8 secretion is platelet-dependent. Whole blood anticoagulated with hirudin was centrifuged at 180 g for 15 min and PRP was removed. 500 μ l of the remaining blood cell fraction was recombined with 500 μ l of autologous PRP or PPP and the 1 ml samples were incubated with LPS (5 ng/ml), saline (control) or epinephrine (1, 10, 100 μ M) for 2 h at 37 $^{\circ}$ C and 160 rpm. At the end of the incubation time 100 μ l 2% EDTA was added and PPP was isolated and frozen at -70 $^{\circ}$ C. IL-8 was quantified using an ELISA Biotrak kit. Results are expressed as means with SEM, n = 3. Values were considered significantly different in the case of $p < 0.05$ by paired Student's t-test (* = $p < 0.05$ compared to the same sample without platelets)

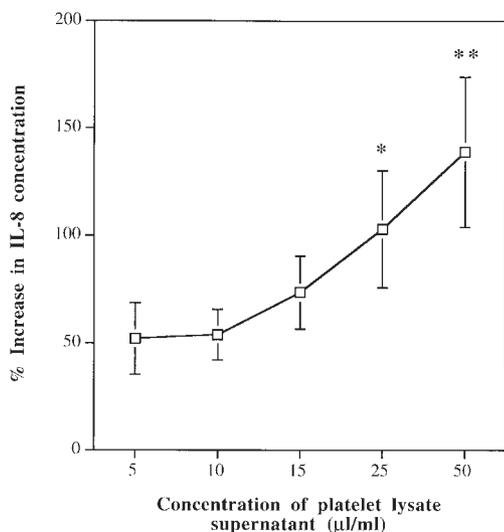


Fig. 5 Platelet lysate supernatant increases LPS induced IL-8 production in whole blood. 1 ml samples of whole blood anticoagulated with heparin were incubated with LPS (5 ng/ml), and increasing concentrations of platelet lysate supernatant (5 µl/ml, 10 µl/ml, 15 µl/ml, 25 µl/ml, 50 µl/ml) for 2 h at 37° C and 160 rpm. After incubation 100 µl 2% EDTA was added and PPP was isolated and frozen at -70° C. IL-8 was quantified using an ELISA Biotrak kit. IL-8 concentration in the control sample (whole blood with 5 ng/ml LPS) was 1518 ± 58 pg/ml IL-8. Results are presented as means with SEM, n = 5. Values were considered significantly different in the case of $p < 0.05$ by the Scheffe F-test of post hoc comparison of means (* = $p < 0.05$, ** = $p < 0.001$)

10⁶ cells/ml) were incubated with 0.2 ng/ml LPS and platelet lysate supernatant for 2 h at 37° C. 15 µl platelet lysate supernatant increased LPS induced IL-8 production by granulocytes with 59%. Platelet lysate *per se* had no effect on IL-8 production in the absence of LPS (data not shown). However, LPS stimulated granulocytes secreted only 10% of the amount of IL-8 secreted by LPS stimulated mononuclear cells (259 ± 31 pg/ml versus 2736 ± 291.2 pg/ml per 10⁶ cells).

Since we have shown in a previous study that the α-granule protein PF4 increased LPS induced TF activity in monocytes (15), we questioned whether the observed effect of lysed platelets could be caused by PF4. As carboxyterminal peptides of PF4 are known to mimic the action of the native molecule (22), a carboxyterminal tridecapeptide of PF4, PF4(58-70), was synthesized. Fig. 7a shows that PF4(58-70) had a strong and significant effect on LPS induced IL-8 production in whole blood (global p -value = 0.00001). 10 µg/ml PF4(58-70) increased IL-8 levels in whole blood with 60%, while 50 µg/ml caused a 115% increase. Next, a mononuclear cell suspension was incubated with LPS and increasing concentrations of PF4(58-70). As shown in Fig. 7b, 1 µg/ml PF4(58-70) had no or a slightly decreasing effect on IL-8 secretion in mononuclear cells, and 5 µg/ml PF4(58-70) tended to increase IL-8 levels, whereas 20 µg/ml PF4(58-70) potentiated IL-8 production with 40%. PF4(58-70) *per se* had no effect on IL-8 production in the absence of LPS (data not shown).

The Effect of Epinephrine on LPS Induced MCP-1 Production in Whole Blood

To see if epinephrine could have any impact on the secretion of another important monocyte-derived chemokine, MCP-1, which is

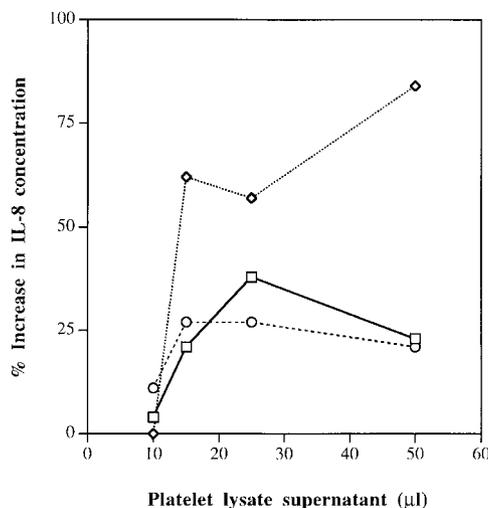


Fig. 6 Platelet lysate supernatant increases LPS stimulated IL-8 production in mononuclear cells. 0.5 ml samples of mononuclear cells (2×10^6 cells/ml) were incubated with LPS (0.2 ng/ml), and increasing concentrations of platelet lysate supernatant (10 µl/ml, 15 µl/ml, 25 µl/ml, 50 µl/ml) for 2 h at 37° C and 160 rpm. The mononuclear cell preparation was contaminated with 19×10^6 platelets/ml. After incubation 50 µl 2% EDTA was added and PPP was isolated and frozen at -70° C. IL-8 was quantified using an ELISA Biotrak kit. Results using cells from three donors are presented. The IL-8 level in the control sample consisting of mononuclear cells stimulated with 0.2 ng/ml LPS was 5239 ± 951 pg/ml (average of 3 experiments). Values were considered significantly different in the case of $p < 0.05$ by the Scheffe F-test of post hoc comparison of means

known to be produced in response to LPS (reviewed in 23), we tested the effect of epinephrine on LPS induced MCP-1 production. Whole blood was stimulated with epinephrine (100 µM) and 5 ng/ml LPS and samples were taken at 0, 1, 2, and 4 h of incubation (Fig. 8). The MCP-1 production in response to LPS started first at 150 min and was delayed in comparison to that of IL-8, which could already be detected at 40 min of incubation (Fig. 1). In contrast to IL-8 production, LPS induced MCP-1 production was inhibited by epinephrine: at 240 min epinephrine reduced MCP-1 levels with 37% ($p = 0.03$). Epinephrine *per se* had no effect on MCP-1 production.

Discussion

In this study we report that epinephrine increases LPS induced IL-8 production in whole blood. As shown in Fig. 1, epinephrine dose-dependently increased LPS induced IL-8 secretion in whole blood. The lowest epinephrine concentration used (0.001 µM) tended to stimulate LPS induced IL-8 production only slightly, whereas 1, 10 and 100 µM potentiated LPS induced IL-8 production significantly. Epinephrine *per se* (100 µM) had no effect on IL-8 production (Fig. 2). In another series of experiments (Fig. 3) it was also shown that epinephrine amplified the generation of IL-8 in blood costimulated with LPS/TNFα and with LPS/PMA. The enhancing effect of epinephrine on LPS induced IL-8 generation described by us seemingly contradicts the reports by several groups who observed a dampening effect of epinephrine on the cytokine response of LPS activated monocytes. According to Sekut et al. (5), the β₂-adrenoceptor agonist salmeterol attenuated LPS induced TNFα production in THP-1 cells, probably by increasing intracellular cAMP levels. Epinephrine also inhibited LPS

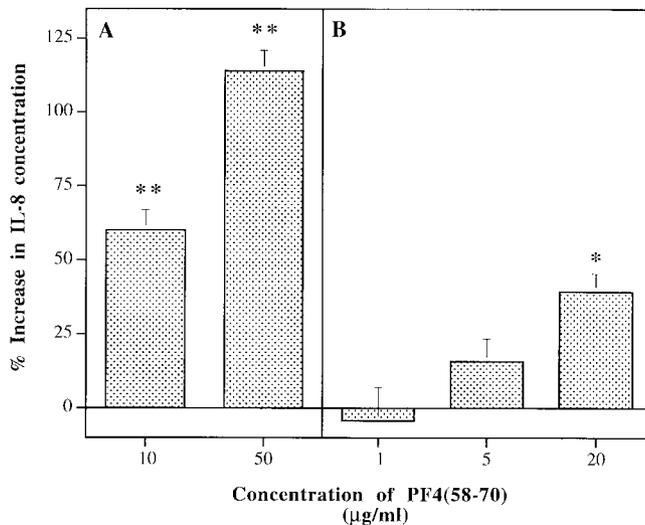


Fig. 7 PF4(58-70) increases LPS induced IL-8 production in whole blood and in mononuclear cells. A) 1 ml samples of whole blood anticoagulated with heparin were incubated with LPS (5 ng/ml) and with PF4(58-70) (10 µg/ml, 50 µg/ml) for 2 h at 37° C and 160 rpm. After incubation 100 µl 2% EDTA was added and PPP was isolated and frozen at -70° C. IL-8 was quantified using an ELISA Biotrak kit. The IL-8 concentration in the control sample (whole blood + 5 ng/ml LPS) was 3100 ± 470 pg/ml. Results are presented as means with SEM, n = 5. Values were considered significantly different in the case of p < 0.05 by the Scheffe F-test of post hoc comparison of means (* = p < 0.05, ** = p < 0.001). B) 0.5 ml samples of mononuclear cells (2.2 × 10⁶ cells/ml) were incubated with LPS (5 ng/ml) and increasing concentrations of PF4 (58-70) (1 µg/ml, 5 µg/ml, and 20 µg/ml) for 2 h at 37° C. The mononuclear cell preparation was contaminated with 19 × 10⁶ platelets/ml. After incubation 100 µl 2% EDTA was added and PPP was isolated and frozen at -70° C. IL-8 was quantified using an ELISA Biotrak kit. The IL-8 concentration in the control sample (mononuclear cells + 5 ng/ml LPS) was 10183 ± 1097 ng/ml. Results are presented as means with SEM, n = 5. Values were considered significantly different in the case of p < 0.05 by the Scheffe F-test of post hoc comparison of means (* = p < 0.05)

stimulated TNF α production in whole blood *ex vivo* by negatively affecting translation (6). Van der Poll et al. (7) observed that infusion of epinephrine in human volunteers 3 h before infusion of LPS resulted in not only a decrease in TNF α levels, but also tended to reduce IL-6 and IL-8 levels. These reports are consistent with our own observations that epinephrine inhibited LPS induced monocyte-associated TF expression in whole blood (Østerud, unpublished), and that epinephrine attenuated LPS induced secretion of the monocyte activation product MCP-1 in whole blood after 4 h (Fig. 8). It may thus be speculated that disparate pathways of activation exist for LPS induced production of monocyte-derived IL-8, MCP-1, TNF α and TF. This is partly supported by a report by Liebler et al. (24) who observed that PHA stimulated monocytes express both IL-8 and MCP-1 although at markedly different rates, as MCP-1 mRNA appears 3 h later than IL-8 mRNA. Furthermore, TNF α *per se* has been shown to induce IL-8 production in monocytes of whole blood (25, Fig. 3), but not TF (26).

Fig. 4 shows that the costimulating effect of epinephrine was dependent on the presence of platelets, as IL-8 production was strongly and significantly increased in samples containing both epinephrine (1–100 µM) and platelets compared to samples lacking platelets (Fig. 4). Epinephrine concentrations ranging from 1 to 100 µM have been commonly used in whole blood studies on platelet activation

(16, 17) and in platelet aggregometry studies (27–29). The enhancing effect of epinephrine on LPS induced IL-8 production in whole blood is probably due to its enhancing effect on platelet activation. Epinephrine stimulates phosphorylation of P-selectin in platelets (30) and has been reported to induce platelet fibrinogen receptor expression, fibrinogen binding and aggregation in whole blood in the absence of other agonists (16). Epinephrine has also been shown to enhance platelet aggregation under moderate levels of shear stress (31), to cause platelet activation *in vivo* measured by filtragometry (32), and to significantly increase plasma β -thromboglobulin levels in hypertensive men (33). Whether epinephrine has any effect on platelet α -granule secretion in normotensive subjects remains controversial, since some groups have measured a significant increase in plasma β -thromboglobulin levels in response to low epinephrine doses (32), whereas others have not (28, 33). Despite of the above cited reports on its platelet activating properties, epinephrine is probably not a true platelet agonist, since epinephrine *per se* did not induce platelet activation in ADP-free preparations of human gel-filtered platelets where endogenous stimulation by the arachidonate pathway was prevented (27). Although the role of epinephrine as a true platelet agonist is controversial, it is generally accepted that epinephrine sensitizes platelets to true platelet agonists. Since LPS stimulated whole blood *ex vivo* is far from being a pure system, exogenously added epinephrine may enhance platelet stimulation in response to trace amounts of ADP, thromboxane A₂ (TxA₂) or immune complexes (34).

The stimulating effect of platelets on LPS induced IL-8 production in whole blood was not dependent on intact platelets, as it could be mimicked by platelet lysate supernatant. Platelet lysate supernatant increased LPS induced IL-8 production in whole blood and in a mononuclear cell suspension dose-dependently and significantly (Figs. 5-6). IL-8 antigen detected in the mononuclear cell suspension was most probably secreted by monocytes, not by lymphocytes. Although both

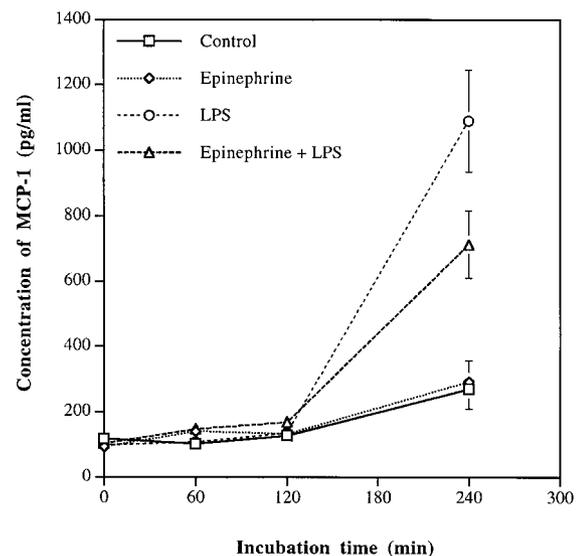


Fig. 8 The effect of epinephrine on LPS induced MCP-1 generation in whole blood. 1 ml samples of whole blood anticoagulated with hirudin were incubated with saline (control), epinephrine (100 µM), LPS (5 ng/ml), and LPS (5 ng/ml) and epinephrine (100 µM) for 60, 120 and 240 min at 37° C and 160 rpm. At the end of the incubation time 100 µl 2% EDTA was added and PPP was isolated and frozen at -70° C. MCP-1 was quantified using an ELISA Quantikine kit. Results are expressed as means with SEM, n = 4. Values were considered significantly different in the case of p < 0.05 by the Scheffe F-test of post hoc comparison of means

monocytes (1, 2) and T-lymphocytes (35) are producers of IL-8, T-lymphocytes (in contrast to monocytes) do not produce IL-8 in response to LPS (36, 37). The current results suggest that one or several platelet products act on monocytes to enhance LPS stimulated IL-8 production. This is confirmed by a study by Weyrich et al. (38) who reported that thrombin activated platelets tethered to monocytes via P-selectin induced expression and secretion of IL-8 as well as monocyte chemotactic protein-1 (MCP-1). The intercellular mediator was presumed to be RANTES, released by activated platelets. The present study points to a role for PF4 as intercellular mediator since we were able to obtain an effect similar to the one observed with platelet lysate supernatant when PF4(58-70) was added to LPS stimulated whole blood or mononuclear cells (Figs. 7a-b). PF4 and PF4(58-70) have been reported to be chemotactic for monocytes (39, 40) and PF4 has been shown to stimulate expression of chondroitin sulphate proteoglycan in human monocytes (41). We have previously shown that platelet lysate supernatant and PF4(58-70) increase LPS induced TF activity in monocytes of whole blood. This was, however, thought to be due to an effect of PF4(58-70) on granulocytes (15).

In conclusion, we demonstrate in this study that epinephrine increases LPS induced IL-8 secretion in human whole blood in a platelet-dependent way. The increasing effect of platelets and platelet contents is suggested to be partly due to PF4. Based on the results obtained in this study and work published by other groups (cited above) we propose the following sequence of events: At the site of inflammation platelets are activated by ADP, TxA_2 and immune complexes. Epinephrine acts to increase platelet adherence to monocytes via P-selectin, as well as promoting the release of PF4 and other platelet α -granule proteins, which potentiate monocyte activation by LPS. Production of the chemoattractant IL-8 by monocytes is upregulated, which in turn leads to enhanced neutrophil infiltration to the inflammatory site.

References

- Walz A, Peveri P, Aschauer H, Baggiolini M. Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. *Biochem Biophys Res Commun* 1987; 149: 755-61.
- Yoshimura T, Matsushima K, Kanaka S, Robinson EA, Apella E, Oppenheim JJ, Leonard EJ. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc Natl Acad Sci USA* 1987; 84: 9233-7.
- Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines - CXC and CC chemokines. *Adv* 1994; 55: 97-179.
- Harada A, Mukaida N, Matsushima K. Interleukin-8 as a novel target for intervention therapy in acute inflammatory diseases. *Mol Med Today* 1996; 2: 482-9.
- Sekut L, Champion BR, Page K, Menius JA, Connolly KM. Anti-inflammatory activity of salmeterol: down-regulation of cytokine production. *Clin Exp Immunol* 1995; 99: 461-6.
- Severn A, Rapson TR, Hunter CA, Liew FY. Regulation of tumor necrosis factor production by adrenaline and β -adrenergic agonists. *J Immunol* 1992; 148: 3441-5.
- van der Poll T, Coyle SM, Barbosa K, Braxton CC, Lowry SF. Epinephrine inhibits tumor necrosis factor- α and potentiates interleukin 10 production during human endotoxemia. *J Clin Invest* 1996; 97: 713-9.
- Watson F, Robinson JJ, Edwards SW. Neutrophil function in whole blood and after purification: Changes in receptor expression, oxidase activity and responsiveness to cytokines. *Biosci Rep* 1992; 12: 123-33.
- van Ginkel GJ, van Aken WG, Oh JI, Vreeken J. Stimulation of monocyte procoagulant activity by adherence to different surfaces. *Br J Haematol* 1977; 37: 35-45.
- Haskill S, Johnson C, Eierman D, Becker S, Warren K. Adherence induces selectively mRNA expression of monocyte mediators and proto-oncogenes. *J Immunol* 1988; 140: 1690-4.
- Hofslie E, Lamvik J, Nissen-Meyer J. Evidence that tumour necrosis factor (TNF) is not constitutively present in vivo. *Scand J Immunol* 1988; 28: 435-41.
- Sporn SA, Eierman DF, Johnson CE, Morris J, Martin G, Ladner M, Haskill S. Monocyte adherence results in selective induction of novel genes sharing homology with mediators of inflammation and tissue repair. *J Immunol* 1990; 144: 4434-41.
- de Groote D, Zangerle PF, Gevaert Y, Fassotte MF, Beguin Y, Noizat-Pirenne F, Gathy R, Lopez M, Dehart I, Igot D, Baudrihaye M, Delacroix D, Franchimont P. Direct stimulation of cytokines (IL-1 β , TNF- α , IL-6, IFN- γ and GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. *Cytokine* 1992; 4: 239-48.
- Kasahara K, Strieter RM, Chensue SW, Standiford TJ, Kunkel SL. Mononuclear cell adherence induces neutrophil chemotactic factor/Interleukin-8 gene expression. *J Leuk Biol* 1991; 50: 287-95.
- Engstad CS, Lia K, Rekdal Ø, Olsen JO, Østerud B. A novel biological effect of platelet factor 4 (PF4): Enhancement of LPS-induced tissue factor activity in monocytes. *J Leuk Biol* 1995; 58: 575-81.
- Shattil SJ, Budzynski A, Scrutton MC. Epinephrine induces platelet fibrinogen receptor expression, fibrinogen binding, and aggregation in whole blood in the absence of other excitatory agonists. *Blood* 1989; 73: 150-8.
- Sanderson HM, Heptinstall S, Vickers J, Loesche W. Studies on the effects of agonists and antagonists on platelet shape change and platelet aggregation in whole blood. *Blood Coagul Fibrinol* 1996; 7: 245-8.
- Anfossi G, Trovati M. Role of catecholamines in platelet function: pathophysiological and clinical significance. *Eur J Clin Invest* 1996; 26: 353-70.
- Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B, Baggiolini M. Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/Interleukin-8. *J Exp Med* 1991; 173: 771-4.
- Altstaedt J, Kirchner H, Rink L. Cytokine production of neutrophils is limited to interleukin-8. *Immunology* 1996; 89: 563-8.
- Aziz KA, Cawley JC, Zuzel M. Platelets prime PMN via released PF4: mechanism of priming and synergy with GM-CSF. *Br J Haematol* 1995; 91: 846-53.
- Zucker MB, Katz IR. Platelet factor 4: Production, structure, and physiologic and immunologic action. *Proc Soc Exp Biol Med* 1991; 198: 693-702.
- Sozzani S, Locati M, Allavena A, van Damme J, Mantovani A. Chemokines: a superfamily of chemotactic cytokines. *Int J Clin Lab Res* 1996; 26: 69-82.
- Liebler JM, Kunkel SL, Burdick MD, Standiford TJ, Rolfe MW, Strieter RM. Production of IL-8 and monocyte chemotactic peptide-1 by peripheral blood monocytes. *J Immunol* 1994; 152: 241-9.
- DeForge LE, Kenney JS, Jones ML, Warren JS, Remick DG. Biphasic production of IL-8 in lipopolysaccharide (LPS)-stimulated human whole blood. *J Immunol* 1992; 148: 2133-41.
- Halvorsen H, Olsen JO, Østerud B. Granulocytes enhance LPS-induced tissue factor activity in monocytes via an interaction with platelets. *J Leuk Biol* 1993; 54: 275-82.
- Steen VM, Holmsen H, Aarbakke G. The platelet-stimulating effect of adrenaline through α_2 -adrenergic receptors requires simultaneous activation by a true platelet stimulatory agonist. *Thromb Haemost* 1993; 70: 506-13.
- Kjeldsen SE, Weder AB, Egan B, Neubig R, Zweifler AJ, Julius S. Effect of circulating epinephrine on platelet function and hematocrit. *Hypertension* 1995; 25: 1096-105.
- Nakamura T, Ariyoshi H, Kambayashi J, Ikeda M, Shinoki N, Kawasaki T, Monden M. Signal transduction system in epinephrine stimulated platelets; comparison between epinephrine sensitive and insensitive platelets. *Thromb Res* 1997; 85: 83-93.
- Crovello CS, Furie BC, Furie B. Rapid phosphorylation and dephosphorylation of P-selectin accompanies platelet activation. *J Biol Chem* 1993; 268: 14590-3.

31. Goto S, Handa S, Takahashi E, Abe S, Handa M, Ikeda Y. Synergistic effect of epinephrine and shearing on platelet activation. *Thromb Res* 1996; 84: 351-9.
32. Larsson PT, Wallen NH, Egberg N, Hjendahl P. α -Adrenoceptor blockade by phentolamine inhibits adrenaline-induced platelet activation in vivo without affecting resting measurements. *Clin Sci* 1992; 82: 369-76.
33. Lande K, Kjeldsen SE, Os I, Westheim A, Hjermann I, Eide I, Gjesdal K. Increased platelet and vascular smooth muscle reactivity to low-dose adrenaline infusion in mild essential hypertension. *J Hypertension* 1988; 6: 219-25.
34. Sims PJ, Wiedmer T. Induction of cellular procoagulant activity by the membrane attack complex of complement. *Semin Cell Biol* 1995; 6: 275-82.
35. Gregory H, Young J, Schroeder JM, Mrowietz U, Christophers E. Structure determination of a human lymphocyte derived neutrophil activating peptide (LYNAP). *Biochem Biophys Res Commun* 1988; 151: 883-90.
36. Strieter RM, Chensue SW, Standiford TJ, Basha MA, Showell HJ, Kunkel SL. Disparate gene expression of chemotactic cytokines by human mononuclear phagocytes. *Biochem Biophys Res Commun* 1990; 166: 886-91.
37. Smyth MJ, Zachariae COC, Norihisa Y, Ortaldo JR, Hishinuma A, Matsu-shima K. IL-8 gene expression and production in human peripheral blood lymphocyte subsets. *J Immunol* 1991; 146: 3815-23.
38. Weyrich AS, Elstad MR, McEver RP, McIntyre TM, Moore KL, Morrissey JH, Prescott SM, Zimmerman GA. Activated platelets signal chemokine synthesis by human monocytes. *J Clin Invest* 1996; 97: 1525-34.
39. Deuel TF, Senior RM, Chang D, Griffin GL, Henrikson RL, Kaiser ET. Platelet factor 4 is chemotactic for neutrophils and monocytes. *Proc Natl Acad Sci USA* 1981; 78: 4584-7.
40. Osterman DG, Griffin GL, Senior RM, Kaiser ET, Deuel TF. The carboxyl-terminal tridecapeptide of platelet factor 4 is a potent chemotactic agent for monocytes. *Biochem Biophys Res Commun* 1982; 1078: 130-5.
41. Uhlin-Hansen L, Langvoll D, Kolset SO. Blood platelets stimulate the expression of chondroitin sulfate proteoglycan in human monocytes. *Blood* 1992; 80: 1058-65.

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