Interleukin-1 Stimulates Prostacyclin Production by Cultured Human Endothelial Cells by Increasing Arachidonic Acid Mobilization and Conversion

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Interleukin-1 (IL-1) induced slow, lasting activation of human endothelial cells (EC) to release prostacyclin (PGI2). This was accompanied by endogenous 3H-arachidonic acid (3H-AA) release and by a time-dependent increase in the cells’ ability to convert exogenous AA. The continuous presence of IL-1 was not required, but about a 1-hour stimulation with the cytokine was sufficient to trigger the cells to synthesize PGI2 for several hours. The spectrum of 3H-AA conversion shows that, in addition to 6-keto-prostaglandin F1α, prostaglandin F2α also was raised after IL-1. The recovery of PGI2 synthesis after aspirin was faster in IL-1-treated EC than in control cells. These data define some of the characteristics of IL-1 stimulation of PGI2 and suggest that this process is mediated both by endogenous AA mobilization and by an increase in cyclooxygenase activity. (Arteriosclerosis 10:129–134, January/February 1990)

Prostacyclin (PGI2) is an important biosynthetic product of endothelial cells (EC). It inhibits platelet aggregation and thrombus formation and acts as a potent vasodilator by causing relaxation in vascular smooth muscle cells.1 PGI2 half-life, when released into the circulation, is in the range of a few minutes, and its activity on platelets and vascular tone is extremely short lived.1 Therefore, its biological relevance in vivo is strictly related to “how much and for how long” it can be produced by the vessel wall.

Most known activators, e.g., thrombin, bradykinin, or ionophore A23187, induce a very fast, short-lasting release of PGI2 by EC, followed by cell refractoriness to subsequent stimulation.2,3 We and other groups have shown that the immunomediator, interleukin-1 (IL-1), has a peculiar way of inducing PGI2 synthesis in EC.4,5 IL-1 stimulation requires a few hours to become apparent, but then it lasts for several hours.

IL-1 is probably one of the most important mediators of the hemodynamic and hematological changes characteristic of septic shock.6,7 The hemodynamic alterations, particularly the hypotension caused by IL-1, require cyclooxygenase products, since they are reversed by inhibitors of this enzyme.7 It has, therefore, been hypothesized that PGI2 synthesis by EC plays an important role in mediating the slow and lasting drop in systemic vascular resistance that follows IL-1 release in the circulation.

Despite its biological importance, little is known about the mechanism and the pattern through which IL-1 exerts its stimulatory activity on EC. In this work, we report that IL-1 induces endogenous arachidonic acid (AA) mobilization and increases its conversion to prostaglandins via an increase in cyclooxygenase synthesis and activity. This effect is long lasting but does not need the continuous presence of IL-1 to be apparent.

Methods

Endothelial Cell Culture

EC were isolated from human umbilical vein and cultured in medium 199 supplemented with 20% newborn calf serum as previously described in detail.2 The cells were used at the first passage at confluence, and they were maintained at 37°C in a water-saturated atmosphere at 95% air/5% CO2 and fed twice a week. EC were routinely characterized by indirect immunofluorescence with rabbit anti-human von Willebrand factor antibodies (Behringwerke AG, Marburg, FRG). All culture reagents were purchased from GIBCO-Europe, Paisley, Scotland. The plastic culture was obtained from Cel-Cult, Flow Laboratories, Irvine, Scotland.

Endothelial Cell Stimulation with Interleukin-1

After removal of the growth medium, the monolayers of intact confluent EC (1.5 to 2×10⁵ cells in a 4 cm² culture well) were washed once with 2 ml of phosphate-buffered saline (PBS). Each well was then incubated with 1 ml of culture medium with IL-1 (Ultrapure IL-1, Genzyme, Boston, MA) added at the required concentration. After selected incubation times at 37°C, the supernatants were removed from the culture wells, were centrifuged for 2 minutes in a microfuge (Beckman Instruments, Fullerton, CA), and were tested for their content of 6-keto-prostaglandin F₁α (6-keto-PGF₁α), the stable PGI₂ metabolite, by a specific radioimmunoassay as previously described in detail.8 The anti-6-keto-PGF₁α antibody used for this assay was a kind...
gift from M.R. Buchanan (McMaster University, Hamilton, Ontario, Canada).

In some experiments at selected times, the medium containing IL-1 (10 U/ml) was removed and tested for 6-keto-PGF$_{1\alpha}$, the cells were washed twice with 2 ml of PBS, and were then incubated with the culture medium for different time periods. The medium was next removed and tested for 6-keto-PGF$_{1\alpha}$.

In other experiments, after incubation with 10 U/ml of IL-1 for selected time periods, the EC were washed with PBS and were challenged with 10 μM of AA (Nu Check Preparation, Elysian, MN) for 15 minutes at 37°C. AA was prepared and stored as described previously. At the end of this period, the supernatant was removed, and the 6-keto-PGF$_{1\alpha}$ content was measured.

In still other experiments, 2 to 15 μM of the ionophore A23187 (Calbiochem, La Jolla, CA) or 5 U/ml of thrombin (Topostasine Roche, Milano, Italy) were added as the stimulus instead of AA, and the same experimental design was followed. In some experiments, hirudin (Laboratoire Stago, Paris, France) at a concentration of 10 U/ml was added to the cells during incubation with 10 U/ml of IL-1.

**Results**

To investigate the mechanism of IL-1 mediated PGI$_2$ synthesis, we first tested whether this cytokine induced endogenous $^{3}$H-AA mobilization from the cell membrane.

Figure 1 reports the time course of IL-1 stimulation of 6-keto-PGF$_{1\alpha}$ and of $^{3}$H-AA release by EC. IL-1 induced slow, but lasting, release of 6-keto-PGF$_{1\alpha}$, and of the radioactivity associated with $^{3}$H-AA. The two curves did not completely overlap. The release of radioactivity reached a plateau between 8 and 24 hours, while the 6-keto-PGF$_{1\alpha}$ levels continued to rise. These experiments were performed in the presence of serum as described in the Methods section. However, comparable results were obtained when 0.25% of bovine serum albumin was substituted for the serum.

The possibility that IL-1 induces 6-keto-PGF$_{1\alpha}$ through a second mediator was also considered. Since IL-1 has been shown to increase EC procoagulant activity, we incubated the cells with IL-1 (10 U/ml) in the presence of hirudin (10 U/ml) for 24 hours. No difference in the amount of 6-keto-PGF$_{1\alpha}$ released in the medium was observed.

We then investigated whether IL-1 could also increase the capacity of EC to metabolize exogenous AA. This was tested at different times after IL-1 activation. As shown in Figure 2A, EC treated with 10 U/ml of IL-1 converted exogenous AA to 6-keto-PGF$_{1\alpha}$ more efficiently than did the control cells. This effect was apparent after a 4-hour incubation with IL-1 and was maximal at 6 hours. In contrast, when EC were treated with A23187 (25 μM for 15 minutes) at different times after IL-1 activation (10 U/ml), no significant increase in the ability of IL-1 treated cells to produce 6-keto-PGF$_{1\alpha}$ was observed over that of control cells (Figure 2B). Comparable results were obtained when the A23187 concentration was decreased to 2 μM; in a typical experiment, control cells produced 7.6±0.6 pmol of 6-keto-PGF$_{1\alpha}$ per 10$^6$ cells (means±SEM of three replicates) in response to 2 μM of A23187, while...
IL-1 treated cells at 24 hours produced 8.0±1 pmol of 6-keto-PGF$_{1\alpha}$ per 10$^5$ cells. In three experiments, thrombin (5 U/ml) was used as a stimulus at 24 hours after IL-1 treatment (10 U/ml). In this case, a significant increase in the 6-keto-PGF$_{1\alpha}$ production at 15 minutes after thrombin stimulus was found in IL-1 treated cells (40.3±5 pmol 6-keto-PGF$_{1\alpha}$ per 10$^6$ cells [means±SEM of three experiments] over that found in control cells (10.8±7 pmol of 6-keto-PGF$_{1\alpha}$ per 10$^6$ cells).

To assess whether the effect of IL-1 on EC was specific for 6-keto-PGF$_{1\alpha}$ and not for other prostaglandins, we studied the spectrum of $^3$H-AA conversion at 7 hours after the addition of IL-1. As shown in Figure 3, the major products of $^3$H-AA conversion were 6-keto-PGF$_{1\alpha}$ and PGF$_{2\alpha}$. Both prostaglandins were significantly increased by IL-1. The pattern of $^3$H-labeled prostaglandins was similar when stimulation was prolonged for 24 hours (data not shown).

To determine whether IL-1 has direct effects on de novo cyclooxygenase synthesis, we studied the kinetics of recovery of 6-keto-PGF$_{1\alpha}$ release after the addition of aspirin, which irreversibly inactivates the enzyme. After aspirin, the control cells released very little 6-keto-PGF$_{1\alpha}$, which did not significantly change up to 8 hours after treatment (Figure 4). In contrast, when EC treated with IL-1 were incubated with aspirin, they recovered about 60% of their ability to synthesize 6-keto-PGF$_{1\alpha}$ by 8 hours after treatment.

IL-1 was present in the culture medium throughout the experiments reported above. We investigated whether the continuous presence of this cytokine was required to continuously stimulate 6-keto-PGF$_{1\alpha}$. As reported in Figure 5, after increasing times of incubation with IL-1 (10 U/ml), EC were washed and cultured in the absence of the cytokine for times up to 24 hours. Figure 5 lists the amounts of 6-keto-PGF$_{1\alpha}$.
which accumulated in the medium during EC incubation with IL-1 and its subsequent removal. One hour's incubation with IL-1 was long enough to trigger a subsequent significant increase in PGI₂ production at 24 hours. Shorter incubation times were ineffective. The maximal total value of 6-keto-PGF₁α at 24 hours was reached when the cells were incubated with IL-1 for at least 5 to 7 hours.

In three experiments after the 24-hour incubation with IL-1 (5 U/ml), the cells were washed twice and incubated for an additional 24 hours with control medium or IL-1 (5 U/ml). The amount of 6-keto-PGF₁α produced at 24 hours after the second IL-1 stimulation (418.1 ± 38 pmol 6-keto-PGF₁α per 10⁵ cells mean ± SEM of three replicates from one typical experiment) was not significantly different from the amount produced at 24 hours after the first stimulation (344.3 ± 41 pmol 6-keto-PGF₁α per 10⁵ cells). Thus, we showed that the cells could be stimulated a second time with IL-1. However, when the cells that have been previously stimulated with IL-1 for 24 hours were cultured with control medium for an additional 24 hours, they produced a higher amount of 6-keto-PGF₁α (93.8 ± 7 pmol 6-keto-PGF₁α per 10⁵ cells) than unstimulated cells (17 ± 7 pmol 6-keto-PGF₁α per 10⁵ cells). This indicates that some of the effect of the first stimulation with IL-1 lasted more than 24 hours and was additive to the effect of the second IL-1 addition.

**Discussion**

IL-1 induces a slow, lasting activation of EC to release PGI₂. Stimulation of PGI₂ synthesis might be mediated by three different mechanisms: 1) mobilization of free AA from membrane phospholipids through activation of phospholipases, 2) increase in cyclooxygenase activity, 3) selective increase in PGI₂ synthetase activity. Considering the mechanism of action of IL-1, the last pathway appears unlikely since IL-1 also stimulated the conversion of AA to PGF₂α, suggesting a more general activation of AA metabolism. This is consistent with IL-1 induced synthesis of other prostaglandins (i.e., PGE₂ or PGF₂α) in other cell types as fibroblasts or condrocytes.⁹,¹⁰,¹¹

The data reported here suggest that IL-1 induces both AA mobilization and an increase in cyclooxygenase activity in EC. The first point is supported by the observation that IL-1 induces time-dependent release of radioactivity from ³H-AA labeled EC. This radioactivity in the culture medium corresponded to free AA and to products of its metabolic conversion. The release of radiolabeled AA reached a plateau between 8 and 24 hours, while PGI₂ synthesis continued to increase. This observation may have some explanation. There may be different pools of AA affected by IL-1, and some endogenous unlabeled AA could be released at different times than the incorporated radiolabeled AA. Alternatively, since cyclooxygenase activity induced by IL-1 (see below and Figure 2A) remained high up to 24 hours, this effect might be sufficient to justify the increased amount of PGI₂ produced between 8 and 24 hours.

The enhanced PGI₂ synthesis from exogenous AA and the increased recovery of PGI₂ synthesis after aspirin in EC treated with IL-1 is consistent with stimulation of cyclooxygenase activity. Recovery from aspirin inhibition is totally dependent on protein synthesis,¹² and the PGI₂ stimulating activity of IL-1 is blocked by protein synthesis inhibitors.⁴ This supports the concept of increased de novo synthesis of cyclooxygenase in EC treated with IL-1.
Other authors have shown in rabbit chondrocytes, in human dermal fibroblasts, and in 3T3 fibroblasts that IL-1 increases phospholipase A2 and cyclooxygenase activity. In 3T3 fibroblasts the IL-1 effect was specific for cyclooxygenase, and PGE2 isomerase was unaffected.

In addition, the IL-1-induced activation of phospholipase A2 appears to be specific and no change in phospholipase C was observed. Other authors, however, showed that protein kinase C activation could act synergistically with IL-1 in increasing AA metabolism.

IL-1 increases both cyclooxygenase activity and the number of enzyme molecules as measured by specific antibody binding. This last effect was due to an increased rate of synthesis of the enzyme.

It has generally been thought that the concentration of free arachidonate released into the cell cytoplasm by phospholipase activation could be the limiting factor in the synthesis of prostaglandins. However, direct and indirect examples showing that an increase in cyclooxygenase activity is accompanied by an augmented production of prostaglandins are now available. These examples include "platelet-derived growth factor" stimulation of PGE2 synthesis in 3T3 cells and thrombokin- and bombesin-induced prostaglandin synthesis from exogenous AA.

An intriguing observation is that IL-1 treated cells were not able to produce more prostacyclin than control cells after A23187 (Figure 2). One possible explanation is that the stimulus was too strong to be further potentiated. However, comparable data were obtained by using threshold concentrations of the ionophore (2 μM). Another possible explanation is that A23187 induces the release of a different pool of endogenous AA, which is converted to PGI2 in a way unaffected by the IL-1 treatment. Interestingly, thrombin activation of the cells appears to be potentiated by the previous treatment with IL-1. This last observation is in agreement with what has been shown in 3T3 fibroblasts after stimulation with bradykinin, thrombin, and bombesin treatment. In these cells, the effect of IL-1 on these stimuli was attributed, at least in part, to induction of G proteins and a consequent amplification of G protein-coupled receptors reactions.

Other mechanisms of action of IL-1 in inducing PGI2 could be considered as an indirect effect through a second mediator. IL-1 has been reported to stimulate human EC to produce thromboplastin activity and "platelet activating factor." Thrombin, however, does not seem to be involved, since activation of the cells in the presence of a high concentration of hirudin does not change the response. In addition, in our laboratory, platelet activating factor did not increase PGI2 production by human EC.

This study also found that IL-1 did not have to be present continuously to induce a significant increase in PGI2 synthesis by EC. The data reported here indicate that about a 1-hour incubation with IL-1 was enough for the cells to be activated for several hours to produce increased amounts of PGI2. Thus, IL-1 appears able within the first period of incubation to trigger a sequence of intracellular events, which then lead to a lasting increase
in the cells' PGJ₂ biosynthetic capacity. This has biological relevance considering that in vivo the hemodynamic effects of IL-1 last much longer than its possible half-life in the circulation and time of contact with the cells. Interestingly, we report here that 24 hours after the first addition of IL-1, the cells can be resensitized by IL-1 to produce PGJ₂. This further underlines the possibility of a prolonged production of this prostaglandin by IL-1-activated cells.

In conclusion, IL-1 belongs to a group of stimuli-like endotoxin, phorbol esters, or platelet-derived growth factors, that induce a long-lasting stimulation of AA metabolism. The activity of these agents requires protein synthesis and, for some of them, may be explained by an increased synthesis of the enzymes responsible for AA conversion. This pattern of activation of prostaglandin, and in particular of PGJ₂, synthesis is different from the other known triggers, such as thrombin, A23187, bradykinin, or polylysine, which induce a rapid conversion of AA that declines within a few minutes and leaves the cells refractory to further stimulation. It is tempting to speculate that this may have different biological meanings in vivo. Considering the extremely short half-life of PGJ₂, a slow, but long-lasting, release of this prostaglandin would promote a sustained vasodilation and eventually a long-lasting inhibition of platelet deposition. A fast, but short, release of PGJ₂ followed by cell refractoriness to further stimulation, might have an acute and marked effect, which could be then rapidly neutralized.

In general, the ability of IL-1 to initiate prostaglandin synthesis is perhaps one of its most important biological properties, accounting for many local and systemic effects, including fever, inflammatory reactions, and hypotension. The way that IL-1 induces prostaglandin synthesis is consistent with its general ability to activate cellular metabolism and induce the expression of genes coding for biologically active molecules.

References

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