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SPECIAL REPORT

Modulation of the induction of nitric oxide synthase by eicosanoids in the murine macrophage cell line J774

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The effect of eicosanoids on the induction of nitric oxide synthase in the murine macrophage cell line J774 has been studied. We found that prostaglandin E_2 (PGE_2) and iloprost (a stable analogue of prostacyclin) both at nanomolar concentrations inhibited the lipopolysaccharide stimulated induction of NO synthase. In contrast $PGF_{2\alpha}$, U46619, a stable analogue of thromboxane A_2, leukotrienes B_4 and C_4 had no effect. These data demonstrate that the L-arginine: NO pathway in macrophages may be modulated by prostanoids.

Keywords: J774 macrophages; eicosanoids; nitric oxide synthase; lipopolysaccharide

Introduction Macrophages activated with bacterial lipopolysaccharide (LPS) express inducible nitric oxide (NO) synthase and produce large amounts of NO which has been reported to be an effector molecule of the cytostatic/cytotoxic properties of these cells (Hibbs *et al.*, 1988).

Macrophages are also an important source of eicosanoids which may modulate their activation state. Thus the cytostatic/cytotoxic effect of activated macrophages appears to be inhibited by some cyclo-oxygenase products like prostaglandin E_2 (PGE_2) and PGI_2 and increased by lipoxygenase metabolites like leukotriene B_4 (LTB_4) and LTC_4 (Bonta & Ben-Efraim, 1987).

In the light of these findings we decided to investigate the effect of eicosanoids on the induction of NO synthase in J774 macrophages.

Methods The murine monocyte/macrophage cell line J774 (American Tissue Culture catalogue T1B 67 page 231) was grown in Dulbecco's modified Eagle's medium (Gibco) at 37°C as previously described (Di Rosa *et al.*, 1990). These cells were plated in 24 well culture plates (Falcon) at a concentration of 2.5×10^5 cells ml^{-1} and allowed to adhere at 37°C in 5% CO_2/95% air for 2 h. Thereafter the medium was replaced with fresh medium and cells were activated by LPS (0.1 $\mu g\,ml^{-1}$) from *Salmonella thyphosa* (Difco). Then cells were incubated in the presence or absence of various concentrations (see Results) of test compounds: PGE_2, $PGF_{2\alpha}$, LTB_4, LTC_4 (Sigma), U46619 (Cayman Chemicals), or iloprost (Schering). After 24 h nitrite (NO_2^-) and eicosanoid levels were assessed in the culture media.

Nitrites were measured with the Griess reagent as previously described (Di Rosa *et al.*, 1990) and PGE_2, 6-keto-$PGF_{1\alpha}$, LTB_4 and LTC_4 by enzyme-immunoassay (Cayman Chemicals). Results are expressed as nmol (NO_2^-) and pmol (eicosanoids) released by 10^6 cells in 24 h.

Data are expressed as mean ± s.e.mean. Comparisons were made by the unpaired two-tailed Student's *t* test. The level of statistically significant difference was defined as $P<0.01$.

Results The production of NO_2^- by unstimulated J774 macrophages was undetectable (<1 nmol per 10^6 cells in 24 h). Incubation of the cells with LPS (0.1 $\mu g\,ml^{-1}$) caused a substantial release of NO_2^- (60.5 ± 1.9 nmol). The basal levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ were 0.44 ± 0.03 pmol and 0.6 ± 0.04 pmol respectively. When the cells were incubated with LPS the levels rose to 8 ± 0.4 pmol for PGE_2 and 11.6 ± 0.5 pmol for 6-keto-$PGF_{1\alpha}$. The level of LTB_4 and LTC_4 in the culture media of unstimulated cells was 0.28 ± 0.03 pmol for LTB_4 and 0.16 ± 0.05 pmol for LTC_4. Both these levels remained unchanged following stimulation of the cells with LPS.

When J774 macrophages were stimulated with the same amount of LPS in the presence of 0.0001–1 μM PGE_2 or of iloprost, a stable PGI_2 analogue, a concentration-dependent inhibition of NO_2^- generation was observed (Figure 1). Iloprost was approximately 10 times more potent that PGE_2. Neither compound at any of the concentrations tested affected NO_2^- generation when added to the cells 6 h after LPS challenge (Figure 1). In contrast, $PGF_{2\alpha}$, U46619 (a stable analogue of thromboxane A_2, TXA_2), LTB_4 and LTC_4, all tested at concentrations ranging from 0.01 to 1 μM, did not significantly affect NO_2^- release when added concomitantly with LPS or 6 h later (data not shown).

Discussion Our results show that J774 macrophages exposed to LPS produce large amounts of both NO_2^- and arachidonic acid metabolites generated by the cyclo-oxygenase pathway, whereas the levels of lipoxygenase pathway metabolites are not affected. These results are in agreement with previously reported data (Humes *et al.*, 1982; Di Rosa *et al.*, 1990).

Here we present evidence that PGE_2 and PGI_2 (iloprost), both at nanomolar concentrations, inhibit LPS-induced

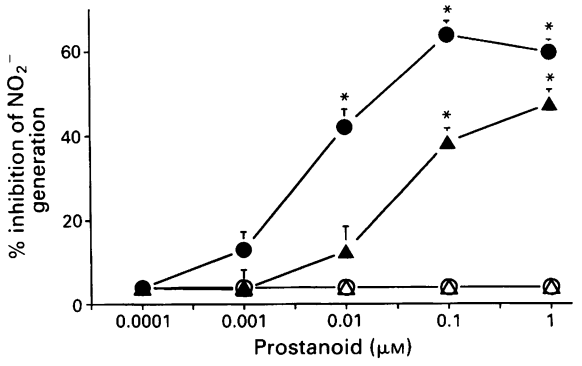

Figure 1 Effect of prostaglandin E_2 (PGE_2) and iloprost on NO_2^- production by lipopolysaccharide (LPS)-activated J774 cells. Solid symbols refer to experiments in which PGE_2 (▲) or iloprost (●) were added to the culture medium concomitantly with LPS. Open symbols refer to experiments in which PGE_2 (△) or iloprost (○) were added to the culture medium 6 h after LPS. The vertical bars show standard errors ($n = 6$).
*$P<0.01$.

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NO_2^- production by J774 cells. Interestingly the concentrations of these eicosanoids which inhibit NO_2^- production are in the same range of those we found in the culture medium of LPS-stimulated J774 cells. Both PGE_2 and iloprost act in a concentration-dependent fashion as inhibitors of the NO synthase induction process since they do not directly inhibit the activity of the enzyme once it has been expressed. Other prostanoids like $PGF_{2\alpha}$ and U46619 did not modify either the induction of NO synthase or the activity of the enzyme.

Nitric oxide has been identified as an effector molecule of the cytotoxic properties of activated macrophages (Hibbs *et al.*, 1988). Our finding of the inhibitory action of PGE_2 and PGI_2 on the induction of NO synthase by LPS is consistent with previously reported observations showing that nanomolar concentrations of PGE_2 inhibited the LPS-induced tumouricidal activity of mouse resident peritoneal macrophages (Taffet *et al.*, 1981).

We could not detect any significant action of exogenously added LTB_4 and LTC_4 on NO_2^- generation by LPS-stimulated J774 macrophages. Our results are in agreement with other studies suggesting that products of the lipoxygenase pathway do not modulate macrophage tumouricidal activity (Schultz *et al.*, 1985). However since other studies have shown that LTB_4 and LTC_4 both increase macrophage tumouricidal activity (Bonta & Ben-Efraim, 1987) our results may suggest that these eicosanoids may not modulate the cytotoxic effect of activated J774 cells.

Our experiments do not explain the mechanism by which PGE_2 and PGI_2 inhibit the induction of NO synthase in J774 cells. Since it is well known that both PGE_2 and PGI_2 stimulate adenylate cyclase in activated macrophages, resulting in an increase in adenosine 3';5'-cyclic monophosphate (cyclic AMP) levels, the mechanism of action of these prostanoids in the regulation of NO synthase induction could be related to this cyclic nucleotide. This hypothesis is supported by the fact that both $PGF_{2\alpha}$ and U46619, which do not enhance cyclic AMP levels, have no action on NO generation. Furthermore, it is interesting to note that nanomolar concentrations of PGE_2, which we have shown to inhibit LPS-induced NO_2^- generation, also inhibit tumour necrosis factor α (TNFα) release from LPS-activated murine macrophages (Renz *et al.*, 1988) and rat Kupffer cells (Peters *et al.*, 1990). Since it has been shown that TNFα induces NO synthase in murine macrophages (Drapier *et al.*, 1988) the inhibition by prostaglandins of the release of TNFα and probably other cytokines could also be considered as a possible explanation of our findings. In this respect the relationship between eicosanoids and the L-arginine: NO pathway, as well as the relevance of this interaction in the host defence mechanism, deserves further investigation.

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