ARACHIDONIC ACID METABOLITES REGULATE INTERLEUKIN-1 PRODUCTION

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We have investigated the role of arachidonic acid metabolites in the regulation of interleukin-1 production by murine peritoneal macrophages. Indomethacin a potent inhibitor of prostaglandin synthesis caused a dose-dependent augmentation of lipopolysaccharide induced interleukin production (up to 7-fold at 5 µM). In contrast, lipoxygenase inhibitors, nordihydroguarietic acid and nafazatrom had no effect at doses that did not significantly decrease prostaglandin synthesis. Added to lipopolysaccharide stimulated cultures, PGE_2 suppressed interleukin in a dose-dependent manner. Zymosan induced interleukin was also augmented by indomethacin but unlike lipopolysaccharide treated cultures was suppressed by nordihydroguarietic acid. These data suggest that arachidonate metabolites may be potent autoregulators of macrophage inter-leukin-1 production. © 1985 Academic Press, Inc.

Interleukin-1 (IL-1)[*] is being increasingly recognized as a important

macrophage/monocyte-derived cytokine with effects on a wide variety of target

tissues. Some of these effects include induction of fever (1), lymphocyte

proliferation/maturation (2,3), the acute phase response (4), muscle catabolism

(5) and fibroblast proliferation (6).

While it is well known that numerous stimuli (e.g. endotoxin, immune

complexes, phagocytosis etc) can induce IL-1 production by macrophages (7,8)

there is little information regarding the factors which regulate its produc-

tion. Considering the physiologic importance of this cytokine, especially in

inflammatory processes, such information might ultimately aid the therapeutic

management of inflammatory disease.

In the present paper we show augmentation of lipopolysaccharide (LPS)-

induced IL-1 production by the cyclooxygenase inhibitor, indomethacin (INDO)

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[*]Abbreviations used in this paper:
    AA, arachidonic acid; IL-1, interleukin-1, INDO, indomethacin; NGDA, nordihydroguarietic acid; LPS, lipopolysaccharide; CO, cyclooxygenase; LO, lipoxygenase.

and direct suppression by exogenously added prostaglandin PGE_2. zymosan-induced IL-1 production was also augmented by INDO. Thus, we provide evidence for a model in which cyclooxygenase derived arachidonic acid metabolites act as feedback regulators of IL-1 production.

MATERIALS AND METHODS

Animals - Female, specific pathogen-free CBA/J mice (Jackson Laboratories, Bar Harbor, ME) were used in all experiments. Mice were maintained under pathogen-free conditions and given food and water ad libitum.

Macrophage cultures - Mouse resident peritoneal macrophages were obtained by peritoneal lavage with sterile RPMI-1640 (Grand Island Biological Co., Grand Island, NY). Cells were washed, counted, then suspended to 1×10^6/ml RPMI with 5% fetal bovine serum (FBS) and 100 U penicillin and 100 µg strepto-mycin/ml. One ml of suspension was dispensed onto 35 mm sterile plastic culture dishes (Corning Glass Works, Corning, NY). After 2h incubation, (37°, 5% CO_2, 100% humidity) nonadherent cells were removed by two vigorous rinses. The monolayers were overlaid with 1 ml of serum-free RPMI with antibiotics plus the agent being tested for IL-1 induction, zymosan (500 µg) or Salmonella lipopolysaccharide (10 µg) (Sigma Chemical Co., St. Louis, MO). Supernates were collected after 18h incubation. The total number of macrophages was quantitated by scraping cells from culture dishes and counting in a hemacytome-ter. These were >95% pure by morphology and phagocytic index.

Arachidonic acid metabolites and metabolic inhibitors - Prostaglandins were kindly provided by Dr. J. Pike (Upjohn Co., Kalamazoo, Mich.). Purity of those compounds was checked by high pressure liquid chromatography performed as previously described (9).

Indomethacin and nordihydroguariatic acid were purchased from Sigma. Nafazatrom was kindly provided by Miles Laboratories, Elkhart, Ind. The in vitro specificity of these compounds in the inhibition of cyclooxygenase and lipoxygenase pathway has been previously published (10).

Radioimmunoassay for prostaglandins - The macrophage culture medium was collected, centrifuged at 500 xg for 10 min and then subjected to radioimmuno-assay for PGE_2 and 6-keto $PGF_{1\alpha}$ as described by Fitzpatrick et al. (11,12). Briefly, samples were extracted to remove protein and free fatty acids before addition of antibodies. Bound and free ligand were separated using dextran-coated charcoal. Sensitivity for PGE_2 and 6-keto $PGF_{1\alpha}$ were 8 and 4 picograms, respectively.

Interleukin-1 assay - Interleukin-1 was measured by the standard thymocyte proliferation assay. Briefly, 5×10^5 mouse thymocytes in 0.1 ml RPMI-10% FBS containing 5 µg/ml PHA (Burroughs Welcome, Research Triangle, NC) were distrib-uted into each well of a 96-well sterile culture dish (Costar, Cambridge, Mass.). Equal volumes of serially diluted test supernate were then added to triplicate wells. Control wells contained no PHA. At 66h of incubation, the cells were pulsed with 0.5 µCi of tritiated thymidine then harvested at 72h. Total thymidine incorporation was determined by liquid scintillation spectro-photometry. Augmentation of PHA-induced thymocyte proliferation indicated IL-1 activity.

Statistical analysis - The Student's t-test was used to compare control and experimental groups. Values of $p > .05$ was considered not significant.

RESULTS

Effect of inhibitors of arachidonic acid metabolism on lipopolysaccharide-induced interleukin-1 production. Initially, we compared known inhibitors of cyclooxygenase (CO) and lipoxygenase (LO) pathways of AA metabolism for their
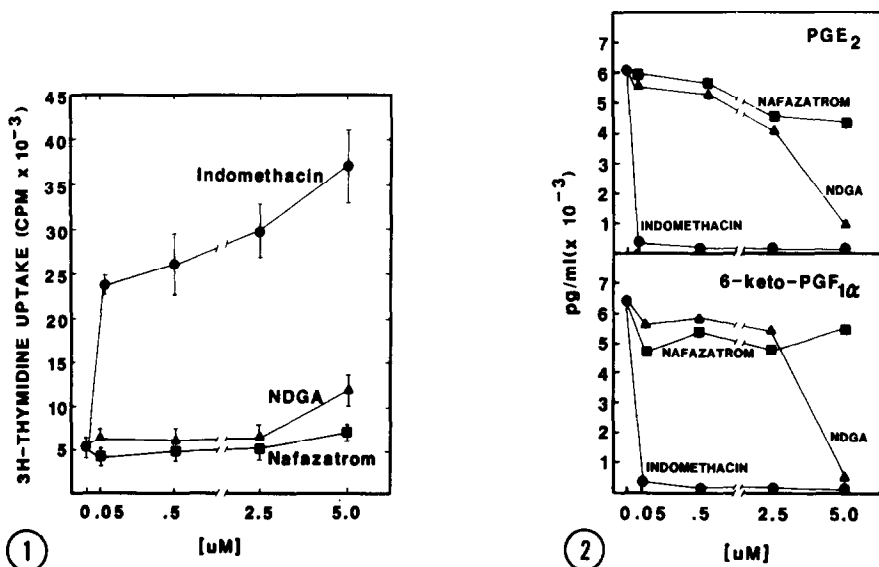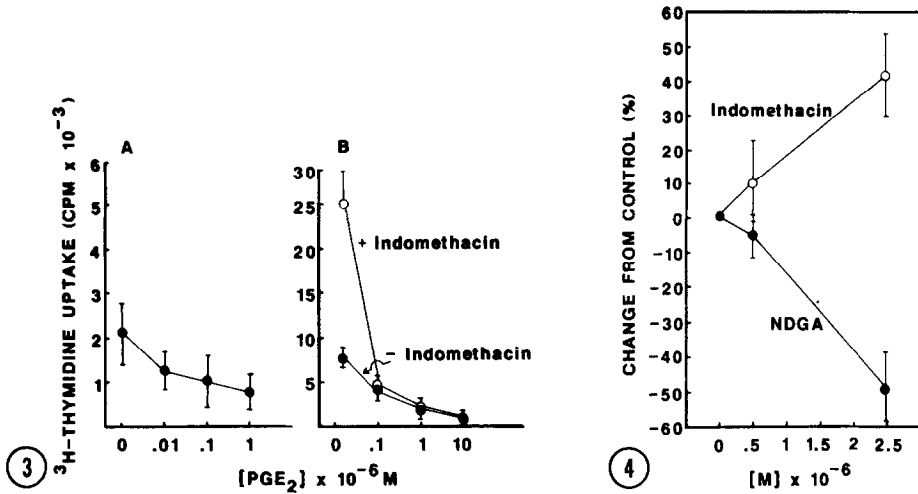
Figure 1.   Effect of inhibitors of arachidonic acid metabolism on LPS-induced IL-1 production. Macrophage cultures (2.2×10^5/dish) were stimulated 18h with LPS (10 µg/ml) in the presence of graded concentrations of inhibitors. Supernates were then tested for IL-1 activity. Control macrophage cultures not stimulated with LPS gave the following values; no inhibitor, 714±401; 5 µM INDO, 773±357; 5 µM NDGA 986±286; 5 µM NAFAZ 699±289. Points are mean CPM ± SD of triplicate determinations.

Figure 2.   Levels of prostaglandins in LPS-stimulated macrophage cultures treated with various inhibitors of arachidonic acid metabolism. The culture supernates described in Figure 1 were tested by radioimmunoassay for PGE_2 and 6-keto-$PGF_{1\alpha}$, a spontaneous breakdown product of PGI_2 (prostacyclin). Points are mean of three determinations.

effect on LPS-induced IL-1 production. Figure 1 shows the dose-response

kinetics of these compounds. A dose-dependent augmentation of IL-1 activity

was obtained with the CO inhibitor, INDO, which caused a 7-fold increase at the

5 µM concentration. In contrast, the LO inhibitors NDGA and nafazatrom had no

significant effect over most of the dose range. However, a 2-fold augmentation

was caused by NDGA at 5 µM. Interestingly, the augmentation of IL-1 activity

was consistently inversely related to the levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ (a

breakdown product of prostacyclin) in the macrophage cultures (Figure 2).

     Effect of exogenous added PGE_2 on interleukin-1 production.   We   next

determined IL-1 production in PGE_2 treated LPS-stimulated macrophage cultures.

As shown in Figure 3 (A & B, closed circles) a dose dependent suppression was

observed. Moreover, the augmentation caused by INDO could be overcome by

Figure 3.   Effect of exogenous PGE$_2$ on LPS-induced IL-1 production. Macrophage cultures were stimulated 18h with LPS (10 µg/ml) in the presence of graded concentrations of PGE$_2$, then supernates were tested for IL-1 activity.   A. Suppression by PGE$_2$, unstimulated control 114±32.   B. Suppression by PGE$_2$ in presence or absence of indomethacin (2.5 µM), unstimulated control 398±172.   Points are mean cpm ± SD of triplicate determinations.

Figure 4.   Effect of arachidonic acid metabolic inhibitors on zymosan-induced IL-1 production.   Percent change in IL-1 activity (^{3}H-thymidine uptake) produced by zymosan-stimulated macrophages in the presence of graded concentrations of indomethacin or NDGA. Points are mean % ± SE of 3-5 experiments.

exogenous PGE$_2$ treatment (Figure 3B, open circles).   Thus, PGE$_2$ could directly

suppress IL-1 production.

   Effect of inhibitors of arachidonic acid metabolism on zymosan-induced

interleukin-1 production.   When we tested CO and LO inhibitors for their effect

on zymosan-induced IL-1 we again saw augmentation by INDO at 2.5 µM (Figure 4).

However, this effect was less dramatic than when LPS was the stimulant.

Furthermore, NDGA caused a definite suppression of zymosan-induced IL-1 at 2.5

µM, an effect not observed when LPS was the stimulant.   These data suggested

that both CO and LO-derived metabolites may play a role in zymosan-induced IL-1

synthesis.

<center>DISCUSSION</center>

   It is well established that macrophages produce significant amounts of

arachidonate metabolites as well as being a major source of IL-1 (13-15).

However, the relationship between these products is not understood.   In the

present report we provide direct and indirect evidence that products of the CO

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pathway (PGE_2, PGI_2) down-regulate the production of IL-1 by macrophages. Thus IL-1 synthesis may be profoundly affected by drugs which inhibit arachidonic acid metabolism (e.g. indomethacin).

Our data imply a model of feedback regulation of IL-1 by prostaglandins. Interestingly, IL-1 can stimulate PGE_2 synthesis in target tissues such as the hypothalamus and muscle (1,5). Hence, it demonstrates the classic characteristic of a hormone by inducing its own inhibitor. It is not clear as yet if IL-1 plays a role in the induction of PG synthesis by macrophages, however studies to resolve this question are currently underway in our laboratory. The auto-regulatory effect of PG on IL-1 production is supported by the finding of Boraschi et al (16) showing that gamma-interferon can augment macrophage IL-1 production by suppression PGE_2 release.

Our finding that IL-1 production was more dramatically augmented by INDO in LPS-stimulated rather than zymosan-stimulated macrophages is most likely related to the differing spectra of AA metabolites induced by these stimuli. Stimulation with membrane perturbants such as LPS causes macrophages to release large amounts of essentially only CO products, PGE_2 and PGI_2, whereas zymosan stimulates production of lesser amounts of PGs as well as some leukotriene C_4 (LTC_4) (17,18). We have confirmed this by high pressure liquid chromatographic analysis of our culture supernates. Furthermore, zymosan is not as potent as LPS in stimulating IL-1 production. Thus, zymosan-stimulated macrophages may be under less tonic suppression by PGs. Moreover, we have recently reported that LTC_4 can directly stimulate IL-1 production (19) and thus may oppose the suppressive effect of PGs.

The potential stimulatory effect of LTC_4 can also explain the observed suppressive effect of NDGA on zymosan-induced IL-1. The synthesis of LTC_4, a lipoxygenase product, is blocked by NDGA. Hence, this would leave the suppressive action of PG unopposed as well as eliminate a stimulus of IL-1 production. It is noteworthy, that Dinarello et al. (2) have reported suppression of human monocyte IL-1 production (induced by a particulate stimulus) by lipoxygenase inhibitors, suggesting these mechanisms may also apply to humans.

In conclusion, we provide evidence that IL-1 production by macrophages is autoregulated by arachidonic acid metabolites i.e. suppressed by PGs. The degree of modulation appears to be affected by the nature of the inducing stimulus and possibly by presence of opposing stimuli such as lipoxygenase pathway metabolites.

ACKNOWLEDGEMENT

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