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Pharmacological modulation of TNF production in macrophages

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The quantity and duration of production of tumor necrosis factor (TNF) is tightly controlled due to its potential to cause serious harm. For example, TNF release in response to overwhelming bacterial infection has been implicated as the first step in potentially lethal septic shock. Prostaglandins and leukotrienes are thought to play opposing roles in regulating TNF production by monocytes and macrophages. We investigated the effects of 5 drugs on the production of TNF by cells of the murine macrophage line RAW264 after stimulation with bacterial lipopolysaccharide endotoxin (LPS). These drugs were of the following 3 classes: cyclooxygenase inhibitors indomethacin (indo) and ibuprofen (ibu); 5-lipoxygenase inhibitors VZ 65 and AA-861; and methylxanthine pentoxyfilline (PTX). While indo and ibu treatment resulted in increased TNF production, PTX, VZ 65, and AA-861 significantly inhibited TNF production, whether administered simultaneously with LPS or 30 min after LPS treatment. VZ 65 and AA-861 also inhibited prostaglandin E_2 (PGE_2) production, coupled with an absence of any rise in intracellular cAMP. Leukotriene B_4 (LTB_4) levels peaked at 15 min and approached background level at 30 min after LPS treatment. Taken together, these data suggest that VZ 65 and AA-861 may inhibit TNF production through mechanism(s) independent of LTB_4 production. VZ 65, AA-861, and PTX all diminished the rate of TNF mRNA transcription, yet VZ 65 and AA-861 appeared to enhance message stability. We conclude that while PTX reduced TNF protein levels by inhibiting TNF mRNA transcription, both VZ 65 and AA-861 exerted opposing effects on TNF transcription and increased mRNA stability.

Key words: Leukotrienes, lipoxygenase inhibitors, macrophages, tumor necrosis factor

Tumor necrosis factor (TNF) is a cytokine with the potential to cause serious harm if either the quantity or duration of its production is excessive. For example, TNF release in response to overwhelming infection by Gram-negative bacteria has been implicated as the first step in septic shock [1]. Consequently, the production of TNF is tightly controlled. Under normal conditions, mRNA coding for TNF is found in trace quantities or not at all in unstimulated monocytes or macrophages. Once induced, this mRNA species is unstable [2]. In addition, investigations have shown that translation of TNF mRNA into protein is also subject to regulation [3,4].

Both prostaglandins and leukotrienes have been reported to modulate TNF production [5,6]. Macrophages and monocytes produce and release prostaglandin E_2 (PGE_2) in response to stimulation with cytokines or lipopolysaccharide endotoxin (LPS), and PGE_2 in turn down-regulates subsequent TNF gene expression [5]. Stimulated macrophages may also metabolize some portion of their membrane-derived arachidonic acid (AA) via the 5-lipoxygenase pathway, resulting primarily in the production of leukotrienes B_4 and C_4 (LTB_4 and LTC_4). It has been reported that inhibitors of 5-lipoxygenase reduced the level of TNF produced by activated rat alveolar macrophages, and that the addition of exogenous LTB_4 restored the TNF level [7]. Blocking production of LTC_4 resulted in a similar reduction of LPS-induced synthesis of TNF from murine peritoneal macrophages [8].

We previously investigated LPS-induced TNF production in the RAW264 murine macrophage cell line resulting from treatment with 5 different pharmacologic agents belonging to 3 classes. The agents investigated

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from the first of these 3 classes, indomethacin (indo) and ibuprofen (ibu), are non-steroidal anti-inflammatory drugs which are potent inhibitors of cyclooxygenase. As such, they block production of all prostaglandins, including PGE_2 [9]. The representative agents from the second class of drugs investigated, the experimental drugs AA-861 (Takeda Corp.) and VZ 65 (Grunenthal GmbH), are inhibitors of 5-lipoxygenase and thus block the leukotriene pathway of AA metabolism. They have also been reported by their manufacturers to inhibit cyclooxgenase, although to a lesser degree. The representative from the third class of drugs investigated, the methylxanthine derivative pentoxifylline (PTX), has been shown to inhibit a membrane-bound phosphodiesterase, leading to increased intracellular levels of cAMP [10]. PTX has also been reported to decrease both phagocytosis and superoxide production by monocytes and neutrophils, through increased intracellular cAMP concentrations in these cells [ll]. We chose the RAW264 macrophage line for these studies because of its well-documented sensitivity to LPS and subsequent regulated production of large quantities of TNF. Furthermore, a cell line provides a simple and uniform system in which to determine key responses, free of confounding influences from lymphocytes or other cell types typically present in primary samples.

Materials and Methods

Cell cultures

RAW264 cells were obtained from the American Type Culture Collection. Culture medium was prepared from powder (GIBCO, Grand Island, NY, USA) reconstituted with clinical grade pyrogen-free distilled water (Travenol, Deerfield, IL, USA), supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan,UT, USA), and sterilized by filtration. No antibiotics were used. Culture medium prepared in this manner and tested routinely with a commercial LPS assay (Whittaker M. A. Bioproducts, Walkersville, MD, USA) did not contain detectable LPS (i.e., <0.01 ng/mL).

Experimental protocols

Cells (5×10^5/mL) were incubated with a dose of LPS (10 ng/mL) that induced measurable amounts of TNF. Culture supernatants were collected at 2, 6, 10, and 24 h after treatment. Drugs were either included in the incubations at the same time as 30 min after LPS was added. At the end of an experiment, culture supernatants were centrifuged, and aliquots of each were saved in sterile polypropylene tubes at –75°C until assayed for TNF, LTB_4, and PGE_2. RAW264 cells were lysed as described below for cAMP measurements or for RNA isolation.

Drugs and LPS

lndo, ibu, and PTX were purchased from Sigma. VZ 65 [4-(ll-hydroxy-l,9-undecadiene)-brenzcatechin] was kindly provided by Grunenthal (Stolberg, Germany). AA-861 [2-(12-hydroxydodeca-5,10-diynyl)-3,5,6-trimethyl-l,4-benzoquinone] was a generous gift from Takeda Chem, Osaka, Japan. LPS (*E. coli* O111:B4) was purchased from Sigma. Dose-response experiments led us to choose the following optimal doses of drugs for these experiments: 5×10^{-7} M VZ 65, 10 μg/mL AA-861 and 5×10^{-4} M PTX. Concentrations of indo and ibu (1×10^{-5} M in each case) and of LPS (10 ng/mL) were adopted from previous reports [12-14].

ELISAs and RIAs

A commercial ELISA system was used for measuring murine TNF (Genzyme, Boston, MA, USA). Levels of PGE_2 and LTB_4 were determined from culture supernatants by RIA (DuPont, Wilmington, DE, USA). Intracellular cAMP was measured by RIA performed on cytosol extracts of lysed cells. Cells were incubated in PBS containing 1 mM isobutyl methylxanthine (IBMX; Sigma-Aldrich, St. Louis, MO, USA) plus LPS and/or drugs at the doses noted above. After 10, 20, and 30 min at 37°C, uncovered dishes were placed in a shallow boiling water bath to kill the cells and evaporate the PBS. Cellular contents were resuspended in sodium acetate buffer supplied with the RIA kit, removed by careful scraping, and assayed according to the manufacturer's instructions.

RNA hybridization analysis

RNA was prepared as previously described [15]. Briefly, total RNA was extracted after guanidinium isothiocyanate (GTC)-mediated cell lysis and purified by ultracentrifugation through CsCl, followed by washing and precipitation of RNA [16]. Purity and quantity of RNA prepared by this method were determined by absorbance of UV light at 260 and 280 nm. RNA was resolved by formaldehyde agarose gel electrophoresis, then transferred to Zetabind nylon membranes for Northern blotting [17]. Equal amounts of each RNA sample (20 μg) were applied to agarose-formaldehyde gels. Blotted RNA was analyzed for

hybridization to the labeled cDNA probe for murine TNF, generously provided by Dr. Arjun Singh, Genentech Corp. The probe was labeled with [^{32}P] deoxycytidine by the random primers method [18]. Labeled blots were visualized by autoradiography and quantified by laser densitometry (Molecular Dynamics software, ImageQuaNT analysis). Subsequently, blots were stripped and reprobed with labeled gamma actin cDNA, a gift of Dr. Tom Maniatis, Harvard University. Densitometric values were also obtained for each actin mRNA signal.

Nuclear run-offs

Run-offs were adapted from a previously described method [19]. Briefly, RAW264 cells were lysed by incubation for 5 min in a buffer containing 10 mM NaCl, 5 mM $MgCl_2$, 20 mM Tris-HCI (pH 7.5), 0.5 mM dithiothreitol, 0.3 M sucrose, and 0.2 % NP-40 detergent. Cells were scraped from culture dishes and centrifuged at 2000 × g for 5 min at 4°C. Nuclear pellets were washed in the lysis buffer and recentrifuged. Pellets were held on ice for 2 min, then added to equal-volume buffered reaction mixtures containing 100 μCi of [α^{32}P]UTP plus unlabeled ATP, CTP, and GTP (l00 μM each). Following a 30-min incubation at 26°C, DNAase I, then proteinase K were added, and the incubation was continued for another 30 min at 37°C. Finally, nuclei were lysed with buffer containing GTC, and RNA was purified using phenol followed by alcohol precipitation. Scintillation counting was performed on an aliquot from each reaction using amounts of RNA containing equal radioactivity to probe strips of nylon membranes to which equal amounts of single-stranded murine TNF or actin cDNAs had been applied using a slot-blotter (BioRad Laboratories, Richmond, CA, USA). Following hybridization, membrane strips were washed and subjected to autoradiography and densitometric scanning as described above.

Statistics

Quantitative measurements from each experimental condition were compared to those from the LPS-only condition using the Student's *t* test. Values of $p<0.05$ were considered significant.

Results

PTX, VZ 65 and AA-861, but not indo or ibu, inhibit production of TNF by macrophages

Tables 1 and 2 summarize the results of experiments

Table 1. Relative TNF levels in RAW264 cells concurrently incubated with LPS and drugs

Condition	2 h	6 h	10 h	24 h
LPS + PTX	0.61 ± 0.10[a]	0.63 ± 0.01[a]	0.55 ± 0.10[a]	0.66 ± 0.08[a]
LPS + VZ-65	0.55 ± 0.13[a]	0.78 ± 0.08	0.69 ± 0.09[a]	0.76 ± 0.08[a]
LPS + AA-861	0.46 ± 0.15[a]	0.71 ± 0.19[a]	0.72 ± 0.09[a]	0.87 ± 0.04[a]
LPS + Indo	1.53 ± 0.10[a]	1.68 ± 0.09[a]	1.66 ± 0.06[a]	1.79 ± 0.08[a]
LPS + Ibu	1.50 ± 0.08[a]	1.79 ± 0.10[a]	1.67 ± 0.10[a]	1.65 ± 0.04[a]

Abbreviations: TNF = tumor necrosis factor; LPS = lipopolysaccharide; PTX = pentoxifylline; indo = indomethacin; ibu = ibuprofen
Note: All values were originally measured as TNF concentrations, in pg/mL, determined in duplicate by ELISA. Values were then normalized relative to the TNF concentration measured in cultures receiving only LPS. TNF in unstimulated control cultures was below detectable levels (<50 pg/mL TNF). Data represent mean ± standard error from 5 separate experiments.
[a]$p<0.05$ compared to LPS alone.

Table 2. Relative TNF levels in RAW264 cells cultures with drugs added 30 min after LPS

Condition	2 h	6 h	l0 h	24 h
LPS; PTX	0.57 ± 0.18	0.61 ± 0.06[a]	0.55 ± 0.04[a]	0.57 ± 0.03[a]
LPS; VZ 65	0.53 ± 0.17[a]	0.69 ± 0.06[a]	0.69 ± 0.06[a]	0.77 ± 0.04[a]
LPS; AA-861	0.58 ± 0.18	0.75 ± 0.07[a]	0.81 ± 0.05[a]	0.77 ± 0.09[a]
LPS; indo	1.69 ± 0.09[a]	1.77 ± 0.08[a]	1.83 ± 0.05[a]	1.68 ± 0.04[a]
LPS; ibu	1.67 ± 0.13[a]	1.68 ± 0.10[a]	1.76 ± 0.10[a]	1.78 ± 0.06[a]

Abbreviations: TNF = tumor necrosis factor; LPS = lipopolysaccharide; PTX = pentoxifylline; indo = indomethacin; ibu = ibuprofen
Note: All values were originally measured as TNF concentrations, in pg/mL, determined in duplicate by ELISA. Values were then normalized relative to the TNF concentration measured in cultures receiving only LPS. TNF in unstimulated control cultures was below detectable levels (<50 pg/mL TNF). Data represent mean ± standard error from 5 separate experiments.
[a]$p<0.05$ compared to LPS alone.

obtained with RAW264 macrophages treated according to the 2 protocols. Table 1 shows the data resulting from concurrent incubation of cells with each drug and LPS. At virtually every point tested, the 5-lipoxygenase inhibitors and PTX, but not the cyclooxgenase inhibitors, significantly decreased LPS-induced TNF production. In the absence of LPS, TNF levels were all below detectable levels (i.e., <50 pg/mL) in supernatants of control cultures and from cells treated only with drugs. LPS-only cultures had mean TNF concentrations ranging from 6.93 to 22.9 ng/mL over the 2- to 24-h incubation intervals. The addition of exogenous PGE_2 (10^{-7} M) with LPS decreased TNF levels to 0.87, 0.65, 0.69, and 0.73 relative to LPS-alone, at 2, 6, 10, and 24 h, respectively. PGE_2 itself induced no TNF production.

VZ 65 and AA-861 decrease PGE_2 levels but PTX does not affect PGE_2

Tables 3 and 4 show the concentrations of PGE_2 measured (by RIA) in separate aliquots of the culture supernatants that produced the TNF values shown in Tables 1 and 2. As expected, the cyclooxygenase inhibitors indo and ibu decreased PGE_2 production significantly. Interestingly, both 5-lipoxygenase inhibitors significantly decreased PGE_2 levels as well, with AA-861 acting with comparable effectiveness to the cyclooxygenase inhibitors. PTX had no significant effect on PGE_2 concentrations. A previous study showed that elevated macrophage production of PGE_2 results in decreased TNF production [20], in agreement with our results in which exogenous PGE_2 was included in the culture (above). The data from the cyclooxygenase inhibitor conditions shown in Tables 1, 2, 3, and 4 confirmed the converse effect, that inhibition of PGE_2 production led to increased TNF levels. However, results obtained with the 5-lipoxygenase inhibitors were paradoxical, with levels of both TNF and PGE_2 significantly decreased. These findings suggest that the 5-lipoxygenase inhibitors use a different mechanism for inhibiting TNF. Results similar to those presented in Tables 1, 2, 3, and 4 were also obtained with the human monocyte line THP-1 (data not shown).

LPS induces a transient increase in LTB_4 levels

Fig. 1 shows the results of a time-course study of LTB_4 measurements obtained between 5 min and 2 h after LPS stimulation. These data show that LPS induced a transient rise in LTB_4 concentrations that peaked at 15 to 20 min.

Table 3. Relative PGE_2 levels in RAW264 cell cultures with drugs added concurrently with LPS

Condition	2 h	6 h	l0 h	24 h
LPS + PTX	0.98 ± 0.09	0.89 ± 0.15	0.94 ± 0.14	0.94 ± 0.14
LPS + VZ 65	0.83 ± 0.12	0.73 ± 0.10[a]	0.63 ± 0.11[a]	0.79 ± 0.06[a]
LPS + AA-861	0.58 ± 0.07[a]	0.37 ± 0.11[a]	0.37 ± 0.14[a]	0.33 ± 0.12[a]
LPS + Indo	0.45 ± 0.07[a]	0.29 ± 0.12[a]	0.27 ± 0.12[a]	0.20 ± 0.08[a]
LPS + Ibu	0.45 ± 0.08[a]	0.29 ± 0.13[a]	0.28 ± 0.12[a]	0.27 ± 0.10[a]

Abbreviations: PGE_2 = prostaglandin E_2; LPS = lipopolysaccharide; PTX = pentoxifylline; indo = indomethacin; ibu = ibuprofen

Note: All values were originally measured as PGE_2 concentrations, in pg/mL, determined in duplicate by RIA. Values were then normalized relative to the PGE_2 concentration measured in cultures receiving only LPS. Data represent mean ± standard error from 5 separate experiments.

[a] $p < 0.05$ compared to LPS alone.

Table 4. Relative PGE_2 levels in RAW264 cell cultures with drugs added 30 min after LPS

Condition	2 h	6 h	l0 h	24 h
LPS, PTX	0.88 ± 0.15	0.94 ± 0.16	0.91 ± 0.13	0.89 ± 0.09
LPS; VZ 65	0.79 ± 0.06	0.66 ± 0.08[a]	0.67 ± 0.09	0.63 ± 0.06
LPS; AA-861	0.51 ± 0.12[a]	0.38 ± 0.15[a]	0.33 ± 0.16[a]	0.46 ± 0.18[a]
LPS; Indo	0.50 ± 0.07[a]	0.29 ± 0.12[a]	0.26 ± 0.12[a]	0.23 ± 0.09[a]
LPS; Ibu	0.65 ± 0.12[a]	0.29 ± 0.11[a]	0.38 ± 0.16[a]	0.31 ± 0.12[a]

Abbreviations: PGE_2 = prostaglandin E_2; LPS = lipopolysaccharide; PTX = pentoxifylline; indo = indomethacin; ibu = ibuprofen

Note: All values were originally measured as PGE_2 concentrations, in pg/mL, determined in duplicate by RIA. Values were then normalized relative to the PGE_2 concentration measured in cultures receiving only LPS. Data represent mean ± standard error from 5 separate experiments.

[a] $p < 0.05$ compared to LPS alone.

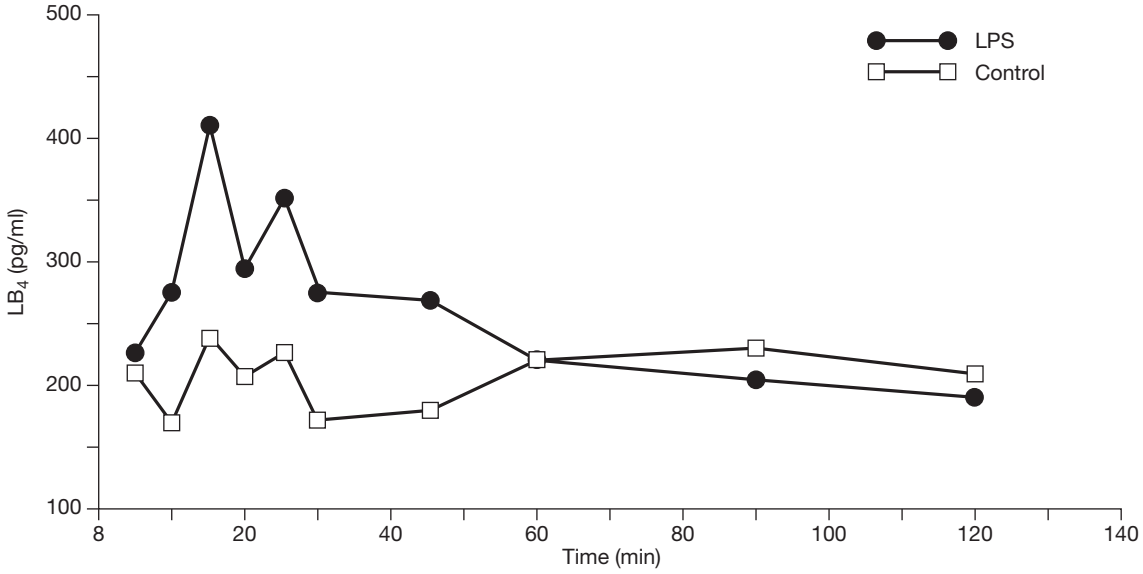

Fig. 1. Time-course of LTB_4 production by RAW264 macrophages. LTB_4 levels were measured in duplicate by RIA in supernatants of cultures after stimulation with 10 ng/mL LPS or in unstimulated controls.

PTX acts transcriptionally: VZ 65 and AA-861 act transcriptionally and stabilize mRNA to inhibit TNF production

To examine the mechanism(s) through which TNF production could be inhibited by VZ 65, AA-861, and PTX, we analyzed steady-state TNF mRNA levels by Northern blotting, and transcription rates by nuclear run-off. Fig. 2 shows a Northern blot of RAW264 RNA probed with radiolabeled TNF cDNA, reflecting concurrent LPS/drug treatments for 2, 6, and 10 h. Densitometric analysis of the resulting autoradiogram showed a slight increase (4 to 9%) in TNF mRNA in cells treated for 2 h with both LPS and any of the drugs, relative to cells treated solely with LPS. Unstimulated controls contained low levels of TNF mRNA at all time points. Later, PTX caused diminished TNF mRNA levels, in agreement with findings of previous reports by others [21,22]. Unexpectedly, treatment with either 5-lipoxygenase inhibitor resulted in increased levels of TNF mRNA at later time points.

Run-offs were performed with cells incubated for 2 h with LPS alone or with LPS plus 1 of the TNF-inhibiting drugs. As shown in Fig. 3, each drug diminished the rate of TNF mRNA transcription compared to LPS treatment alone. Taken together, these findings suggest that PTX treatment simply decreased LPS-induced TNF production by inhibiting transcription of TNF mRNA. In contrast, AA-861 and VZ 65 reduced the transcription rate yet also stabilized existing TNF

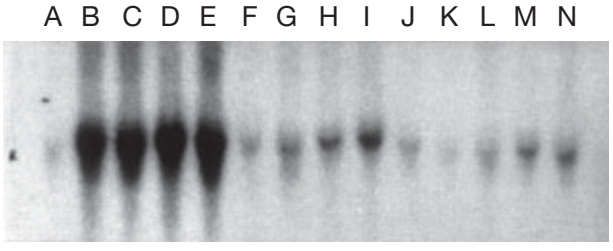

Fig. 2. TNF steady-state mRNA levels. Northern blot of RNA from RAW264 macrophages probed with ^{32}P-labeled TNF cDNA. Each sample contained 20 μg of total RNA. Drugs and LPS were added concurrently. A: unstimulated control, 2 h; B: LPS alone, 2 h; C: LPS + VZ 65, 2 h; D: LPS + AA-861, 2 h: E: LPS + PTX, 2 h; F: unstimulated control, 6 h; G: LPS alone, 6 h; H: LPS + VZ 65, 6 h; I: LPS + AA-861, 6 h; J: LPS + PTX, 6 h; K: unstimulated control, 10 h; L: LPS alone, 10 h; M: LPS + VZ 65, 10 h; N: LPS + AA-861, 10 h.

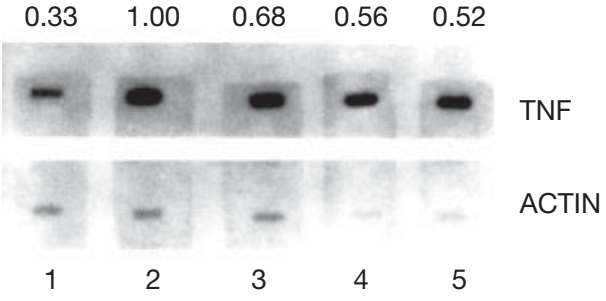

Fig. 3. TNF transcription rates as assessed by nuclear run-off experiment. Numbers above the figure refer to quantity of TNF RNA relative to the LPS-only condition as determined by densitometer. 1: unstimulated control; 2: LPS alone; 3: LPS + VZ 65; 4: LPS + AA-861; 5: LPS + PTX. All incubations were carried out for 2 h.

mRNA, leading to the higher steady-state levels shown in Fig. 2. These observations indicate that AA-861 and VZ 65 affected both transcriptional processes and mRNA stabilization.

In summary, our data indicated that VZ 65, AA-861, and PTX all share the ability to inhibit TNF production, although VZ 65 and AA-861 accomplished this while also causing PGE_2 levels to decrease, a condition usually associated with increased TNF output. In addition, VZ 65 and AA-861 administered 30 min after LPS treatment also effectively inhibited TNF production, even though LTB_4 levels peaked 15 min after LPS treatment and approached background levels at 30 min. Most surprisingly, VZ 65 and AA-861 appeared to stabilize LPS-induced TNF mRNA.

Discussion

TNF production is tightly regulated. In monocytes and macrophages, a stimulus such as LPS is required for appreciable TNF mRNA induction [23]. Once induced, TNF mRNA is relatively unstable [2], and in some cases, is translated into protein with poor efficiency [3,4,24]. Recent reports have shown that AA metabolites play prominent roles in TNF regulation. In macrophages, LPS activates membrane-bound phospholipase A_2, which causes AA release from glycerophospholipid [25]. Cyclooxygenase acts on AA to produce prostaglandins, while 5-lipoxygenase action generates leukotrienes [9]. Products from these 2 branches of AA metabolic pathways are thought to have opposite effects on TNF regulation. Exogenous PGE_2 has been shown to decrease TNF mRNA levels [5,26], while leukotrienes such as LTB_4 and LTC_4 have been reported to increase TNF production [7,8]. Therefore, our findings that the cyclooxygenase inhibitors ibu and indo increased TNF concentrations, while the 5-lipoxygenase inhibitors VZ 65 and AA-861 decreased TNF were consistent with expectations. Similarly, our results that PTX inhibits LPS-induced TNF production were consistent with previous reports [21]. However, our finding that VZ 65 and AA-861 were effective when administered by either protocol was unexpected. Since LTB_4 levels peaked 15 min after LPS stimulation, it was especially surprising that these drugs would inhibit TNF production when administered 30 min after LPS treatment. The time-course of the LPS-induced rise in LTB_4 suggests that VZ 65 and AA-861 inhibit TNF production by a mechanism distinct from that by which they inhibit leukotriene production.

Previous studies have shown that in monocytes and macrophages, elevated levels of PGE_2 activate a membrane-bound adenylate cyclase, resulting in a rise in intracellular cAMP [27]. The increased cAMP has been reported to activate a tetrameric cAMP-dependent protein kinase, resulting in the phosphorylation (and change in activity) of a number of regulatory proteins [28]. Since LPS treatment induces production of both TNF and PGE_2, macrophage-derived PGE_2 is thought to function as an autoregulatory inhibitor, limiting production of TNF [5]. Raising cAMP concentrations by other means has also been reported to result in decreased TNF production [29], whereas inclusion of either indo or ibu in LPS-stimulated cultures of macrophages results in an increase in the production of TNF [30,31]. The rise in TNF concentration and decrease in PGE_2 levels due to treatment with cyclooxygenase inhibitors in this study were therefore consistent with expectations. However, the ability of VZ 65 and AA-861 to decrease PGE_2 concentrations significantly, while also effecting a significant decrease in TNF levels, was unexpected (Tables 3 and 4). This finding suggests that cAMP concentrations might be elevated by an alternative mechanism, negating the effects of decreased PGE_2 levels (especially in the case of AA-861). We measured intracellular cAMP concentrations in unstimulated RAW264 macrophages as well as in cells treated with 10 μM forskolin, LPS alone, and LPS plus VZ 65, AA-861, or PTX for 10, 20, and 30 min. We observed that at 10 min, LPS alone raised the cAMP concentration to 167 ± 49 pg/10^6 cells, which was nearly as high as the level achieved by the forskolin positive control (182 ± 37 pg/10^6 cells). Surprisingly, the drug treatments either had no effect or slightly diminished the LPS-mediated rise in cAMP, although the differences between drug treatments were not significant (data not shown).

We next investigated the mechanism(s) of inhibition of TNF gene expression by VZ 65, AA-861, and PTX. PGE_2-mediated regulation of TNF has been reported to be accomplished by a reduction in the rate at which TNF mRNA is transcribed [26]. Our nuclear run-off data showed that VZ 65, AA-861, and PTX each reduced the rate at which LPS-induced TNF mRNA was transcribed. Steady-state TNF mRNA levels were diminished accordingly, as expected, with PTX treatment [21]. Surprisingly, however, VZ 65 and AA-861 seemed to counteract their negative effects on TNF mRNA transcription with increased stability of the mRNA that was transcribed. Coupled with the drugs' ability to

decrease TNF protein production, these data suggest that VZ 65 and AA-861 also affected translational and/or post-translational processes in these cells. In summary, the 2 experimental 5-lipoxygenase inhibitors appeared to inhibit LPS-induced TNF production by cultured macrophages by mechanisms that affected mRNA transcription, message stability, and some other post-transcriptional process(es).

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