ATP-stimulated Release of Interleukin (IL)-1β and IL-18 Requires Priming by Lipopolysaccharide and Is Independent of Caspase-1 Cleavage*

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Interleukin (IL)-1β and IL-18 are structurally similar proteins that require caspase-1 processing for activation. Both proteins are released from the cytosol by unknown pathway(s). To better characterize the release pathway(s) for IL-1β and IL-18 we evaluated the role of lipopolysaccharide priming, of interleukin-1-β-convertase (ICE) inhibition, of human purinergic receptor (P2X7) function, and of signaling pathways in human monocytes induced by ATP. Monocytes rapidly processed and released both IL-1β and IL-18 after exogenous ATP. Despite its constitutive cytosolic presence, IL-18 required lipopolysaccharide priming for the ATP-induced release. Neither IL-1β nor IL-18 release was prevented by ICE inhibition, and IL-18 release was not induced by ICE activation itself. Release of both cytokines was blocked completely by a P2X7 receptor antagonist, oxidized ATP, and partially by an antibody to P2X7 receptor. In evaluating the signaling components involved in the ATP effect, we identified that the protein-tyrosine kinase inhibitor, AG126, produced a profound inhibition of both ICE activation as well as release of IL-1β/IL-18. Taken together, these results suggest that, although synthesis of IL-1β and IL-18 differ, ATP-mediated release of both cytokines requires a priming step but not proteolytically functional caspase-1.

IL-1β and IL-18 are proinflammatory cytokines that require processing by the IL-1-converting enzyme, caspase-1, at specific aspartic acid residues to generate functional molecules (1–3). Although lacking in sequence homology, IL-1β and IL-18 share significant structural homology (4, 5), bind to receptors from the IL-1 family of receptors (6), exist in the cytosol without a classic signal sequence (3, 7), and are released from cells in a noncanonical fashion (8). Both molecules are regulated posttranscriptionally, and a particularly important aspect of regulation occurs at the level of release (9–14). In this context, recent studies show that this release pathway can be rapidly induced by stimulation with exogenous ATP (15–18). In macrophages, exogenous ATP works via the recently cloned P2X7 receptor, a member of the P2X family of nucleotide-gated channels that are activated by extracellular ATP (19). However, it has been suggested that monocytes do not express P2X7 receptors (20, 21). P2X7 is a 595-amino acid polypeptide with two membrane-spanning domains and intracellular N- and C-terminal domains (22, 23). P2X7 receptor is the only pore-forming P2X family member, and its activation results in the opening of a cationic channel with increased permeability to calcium and intracellular depolarization (24). The present work focuses on release mechanisms by taking advantage of the dramatic ability of ATP to telescope the processing and release down to a 15–30-min interval while enhancing the overall release signal (15).

In this report we examine the mechanism of ATP-induced release of IL-1β and compare it to IL-1β in human monocytes. We show that fresh monocytes express functional P2X7 receptors that modulate IL-1β and IL-18 processing and release in response to ATP. Of note, our data suggest that ATP stimulation alone is insufficient to trigger IL-18 processing and release, since priming with LPS was shown to be necessary. Additionally, the ATP effect on release does not require functional caspase-1, confirming for IL-18 what has been previously shown for IL-1β, i.e. precursor cleavage is not required for release. Finally, attempts to characterize the signaling pathway involved in the ATP effect show that a protein-tyrosine kinase inhibitor, AG126, can potently block activation of caspase-1 and IL-1β/IL-18 release events, whereas mitogen-activated protein kinase, PKC and PKA inhibitors do not.

MATERIALS AND METHODS

Reagents—ProIL-1β-specific rabbit polyclonal antibody (amino acids, 3–21) was developed in our laboratory. Human recombinant IL-1β, biotinylated IL-18, anti-human monoclonal IL-1β antibody (clone 8516), and IL-18 ELISA kit were purchased from R&D Systems (Minneapolis, MN). Mature IL-1β and precursor IL-1β-specific ELISA were from our own laboratory. Polyclonal anti-human P2X7 antibody was from Alomone Laboratories (Jerusalem, Israel), and monoclonal antibody to P2X7 was a gift from Dr. Ian Chessell (University of Cambridge, Cambridge, UK). Recombinant caspase-1 (ICE) and caspase-1 antibody were gifts from Dr. Nancy Thornberry and Dr. Doug Miller (Merck). Herbimycin A, caspase-1 inhibitor (Ac-Tyr-Val-Ala-Asp-chloromethyl ketone (YVAD-cmk)), and tyrphostins and other protein kinase inhibitors were obtained from Calbiochem. Bacterial lipopolysaccharide, Escherichia coli strain 0127:B8, Westphal preparation, was from Difco. LNAME was a gift from Biemol (Plymouth Meeting, PA). Phospho-ERK antibody was obtained from New England Biolab (Plymouth Meeting, PA), and total ERK antibody was from Upstate Biotechnology Inc. (Lake Placid, NY). RPMI 1640 and phosphate-buffered saline were purchased from Biowhittaker Inc. (Walkersville, MD), and fetal bovine serum was from

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‡ The abbreviations used are: IL, interleukin; ICE, interleukin-1-β-convertase; PTK, protein-tyrosine kinase; P2X7, human purinergic receptor; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; PKA and PKC, protein kinases A and C, respectively; ELISA, enzyme-linked immunosorbent assay; YVAD-cmk, acetyl-Tyr-Val-Ala-Asp-chloromethyl ketone; ERK, extracellular signal-regulated kinase; LPS, lipopolysaccharide; PAGE, polyacrylamide gel electrophoresis.

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Hyclone Laboratories (Logan, UT). All other reagents were obtained from Sigma unless otherwise specified.

Isolation of Human Peripheral Blood Monocytes and Cell Culture—Human peripheral blood monocytes were isolated from the heparinized blood of normal donor using histopaque density gradient followed by a monococyte clumping method as described (24). This method yields about 65–75% pure monocytes. Isolated monocytes were cultured at 10^6/ml in RPMI 1640 supplemented with 5% fetal bovine serum at 37°C in humidified incubator. Some cells were cultured for 3 h in the presence of LPS (1 ng/ml) and in the presence or absence of caspase-1 (ICE) inhibitor YVAD-cmk (100 μM).

Preparation of Cell Lysates and Western Blot Analysis—Where indicated, LPS-primed human monocytes were challenged with 5 mM ATP and incubated for an additional 30 min. Culture media was removed from the cells and centrifuged at 800 x g for 5 min, and the supernatants and cell pellets were collected. Cells were lysed in lysis buffer containing 4 mM Tris-Cl, pH 8.0, 150 mM NaCl, 2 mM EDTA, and 1% Nonidet P-40 containing 2 μg/ml leupeptin, aprotonin, chymostatin, pepstatin, antipain, 0.5 mM phenylmethylsulfonyl fluoride, and 50 μM N-[methoxy(sulfonyl)alanyl]-Ala-Ala-Pro-Val chloromethyl ketone. The cell debris and nuclei were removed by centrifugation at 14,000 x g for 20 min. For the analysis of phospho-ERK in cell lysates of monocytes, 1 mM NaVO_3 and 50 mM NaF were included in lysis buffer. The protein concentration in cell lysates was determined using the Bio-Rad protein assay reagent. The proteins were resolved by SDS-PAGE and transferred to nitrocellulose membrane. Nonspecific sites on nitrocellulose membrane were blocked with 5% nonfat dry milk (Carnation) in TBST (25 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) for 3 h at room temperature. For immunoblotting with phospho-ERK antibody, the membrane was blocked with 3% bovine serum albumin in TBST. The membranes were then probed with primary antibodies as indicated followed by peroxidase-conjugated secondary antibodies, and protein bands were visualized by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech).

Flow Cytometric Analysis of P2X_7 Receptors—Isolated monocytes were cultured in complete media alone or in complete media containing LPS (1 ng/ml) for 3 h or overnight in LPS (1 μg/ml). For flow cytometric analysis, cells were stained with monoclonal antibody to P2X_7, essentially as described (25). Briefly, cells were pelleted, washed twice with wash buffer (1% bovine serum in phosphate-buffered saline), and incubated in wash buffer containing 4 μg/ml monoclonal P2X_7, or an isotype (IgG_1) control antibody for 30 min at 4°C followed by fluorescein isothiocyanate-labeled or rhodamine-labeled rat anti-mouse IgG-specific Fab'_2 fragments (Jackson Immunoresearch laboratories, Westgrove, PA). Some cells were also stained for CD14. For the analysis of P2X_7 receptor expression, monocytes were gated for CD14 fluorescence. Flow cytometric analysis was performed using Becton Dickinson FACS Calibur.

IL-1β and IL-18 ELISA—Sandwich ELISAs were developed in our laboratory to detect pro- and mature IL-1β as described (13). The coating antibody has been modified since the previous description. Briefly, anti-human mouse monoclonal IL-1β antibody (clone 8516, R&D Systems) was used as a coating antibody, and rabbit polyclonal pro-IL-1β-specific peptide antibody generated against amino acids 3–21 was used to sandwich the antigen. Horseradish peroxidase (Bio-Rad)-conjugated goat anti-rabbit antibody was used as a developing antibody. The mature IL-1β ELISA used monoclonal antibody clone 8516 and a rabbit polyclonal mature IL-1β antibody (raised against entire 17-KDa mature IL-1β) as coating and sandwich antibodies, respectively. For the measurement of IL-18, ELISA kits were purchased from R&D Systems, and cell associated and released IL-18 contents were determined per manufacturer instructions.

Statistical Analysis—Data are presented as the means ± S.E. of the mean from at least three independent experiments. Simple comparisons were done by paired t test, with p < 0.05 considered to represent statistical significance.

RESULTS

ATP Promotes IL-1β Release—To establish the optimum timing for future analyses of IL-1β processing, we initially examined the kinetics of ATP-induced IL-1β release from LPS-primed human peripheral blood monocytes (Fig. 1). After 3 h of LPS (1 ng/ml), monocytes contained high levels of intracellular IL-1β with only minimal release. However, ATP (5 mM) induced a dramatic release of mature IL-1β, most pronounced between 15–20 min, that began to level off after 30 min of ATP. The accumulation of IL-1β in culture medium correlated with a decrease in cell-associated IL-1β. In all subsequent experiments, ATP treatment was standardized at 30 min.

ATP-stimulated Release of IL-1β and IL-18 Requires LPS Priming—Having established the kinetics of the ATP effect on IL-1β, we next compared the related cytokine IL-18 to IL-1β for ATP-mediated release. As shown in Fig. 2, A and B, the LPS priming induced a dramatic release of both IL-1β and IL-18 at 30 min after ATP treatment. However, there were significant differences in the ability of LPS to induce IL-1β compared with IL-18. Whereas unprimed monocytes contained very little IL-1β intracellularly (Fig. 2C) (2.2 ± 1.5 ng/10^6 cells), low dose LPS induced a dramatic induction of intracellular pro-IL-1β (30.2 ± 12.1 ng/10^6 cells). In contrast, there was no difference between unprimed and LPS-primed monocytes for the presence of intracellular IL-18 (Fig. 2D) (378 ± 22 versus 409 ± 5 pg/10^6 cells, p = not significant).

The constitutive presence of IL-18 in unprimed monocytes provided a unique opportunity to determine whether monocyte IL-18 processing and release induced by ATP requires a priming signal. Of particular note, despite the constitutive presence of intracellular IL-18 in the unprimed monocytes, ATP alone had no effect on inducing its release (Fig. 2B) (control, 54 ± 19 versus ATP, 67 ± 28 pg/10^6 cells). However, after priming with LPS, ATP-induced IL-18 release increased 4-fold (215 ± 60 pg/10^6 cells; p < 0.05 compared with control). Our data thus demonstrate that ATP-stimulated release of IL-18 requires priming by LPS.

Caspase-1 Inhibition Does Not Prevent the Release of IL-1β and IL-18—Caspase-1, which is critical for processing IL-1β and IL-18, has been variably linked to the release pathway for these cytokines. It has been shown for example that caspase-1 −/− macrophages are impaired for IL-1β release (26). To investigate the requirement for caspase-1 function in ATP-induced IL-1β and IL-18 release, we inhibited caspase-1 with the tetrapeptide, YVAD-cmk (100 μM). We analyzed IL-1β and IL-18 release with ELISAs able to detect both cytokines in the precursor form (13). Human monocytes were cultured with YVAD and LPS in varying combinations (Fig. 3). As we and others have previously shown (13, 27), the presence of an ICE inhibitor prevented mature IL-1β release from LPS-primed,
ATP-stimulated, monocytes (data not shown). Importantly, however, a significant quantity of proIL-1β was detected in supernatants using a proIL-1β-specific ELISA (Fig. 3, A and C). As was shown for IL-1β, after LPS priming, ATP induced monocytes to release IL-18 (Fig. 3, B and D). Although ATP alone did not stimulate the release of IL-18 from cells treated with caspase-1 inhibitor, ICE inhibition did not prevent IL-18 release from LPS-primed, ATP-stimulated monocytes (Fig. 3, B and D). These data suggest that release of IL-1β and IL-18 does not require active ICE. Therefore, as has been shown for IL-1β, the processing of IL-18 is not required for its externalization.

**ATP Treatment Promotes IL-1β and IL-18 Processing**—To confirm that the ATP treatment not only induces release but also promotes processing of IL-1β and IL-18, monocyte samples were also assayed by immunoblots (Figs. 4, A and B). We assessed the effects of ATP on the processing of IL-18 and IL-1β in untreated monocytes or in monocytes treated with YVAD or LPS alone or combined LPS and YVAD. Only the 24-kDa precursor form of IL-18 and the 31-kDa form of IL-1β were detectable in cell lysates. In cell supernatants of unstimulated or LPS-primed cells, no IL-1β was detected; however, low levels of the precursor form of IL-18 were detectable. In LPS-primed, ATP-stimulated monocyte supernatants, a large amount of 18-kDa IL-18 was detected. In the same supernatants, the 31-kDa form of proIL-1β was not observed, but a large amount of mature 17-kDa IL-1β was detected.

In the presence of YVAD-cmk, only precursor forms of IL-18 and IL-1β were detected in supernatants of LPS-primed, ATP-treated monocytes (Fig. 4, A and B). There was no obvious difference in the amount of secreted IL-18 and IL-1β in the presence or absence of YVAD. These observations show that YVAD blocks maturation of proIL-1β and IL-18 but does not block the release. Thus, both IL-18 and IL-1β require functional caspase-1 for processing during export.

**ATP Induces ICE Activation**—It has been reported that prolonged ATP treatment (3 h) activates caspase-1 in LPS-primed human monocytes (28). It is not clear whether the short exposure to ATP (30 min) in our experimental system also activates ICE in LPS-primed human monocytes. Therefore, we examined the effect of ATP and YVAD on activation of ICE by immunoblot analysis (Fig. 5A). Monocytes were cultured in the presence or absence of LPS and with or without YVAD for 3 h. ATP (5 mM) was then added where indicated, and cells were incubated for an additional 30 min. In control and LPS-primed cells, the 45-kDa precursor and intermediate forms of ICE were detected, but very little 20-kDa (active form of ICE) was seen.
However, LPS-primed monocytes treated with ATP generated a significantly higher level of mature subunits (p20 and p10) of ICE, which was blocked by the presence of NYAD-cmk.

Since, in the absence of LPS priming, ATP alone had no effect on IL-18 processing and release, it was important to determine whether ATP alone could activate ICE. As shown in Fig. 5B, ATP without LPS activated ICE to the p20 form in both LPS-primed and unprimed monocytes. Thus, the data from ELISA and immunoblots, taken together with the evidence of ICE activation, show that ATP not only activates ICE but also blocks the maturation of these precursor cytokines, it fails to inhibit the release of proIL-1β and proIL-18. Conversely, in the absence of LPS priming, ATP-induced ICE activation does not induce processing and release. Thus, although ATP induces ICE activation, it requires an additional signal to induce processing and release of IL-1β and IL-18.

Monocyte Expression of P2X7 Receptors—A number of reports to date suggest that ATP-induced release of IL-1β in LPS-primed monocytes occurs via P2X7 receptors (15, 25, 29). Human monocytes cultured for 1–3 days in the presence of LPS or monocytes cultured overnight in the absence of any stimulus contain P2X7 receptors (25). However, it is not yet clear if fresh human monocytes cultured for 3 h with or without LPS express P2X7 receptors. To address this question, we analyzed human monocytes cultured in the presence or absence of LPS for 3 h (Fig. 6). Cells were washed with PBS and incubated on ice with 4 μg/ml monoclonal anti-P2X7 antibody for 30 min. The P2X7 receptors were detected with a fluorescein isothiocyanate-labeled donkey anti-mouse F(ab')2 fragment. An IgG2b antibody was used an isotype control. The monocytes were gated by anti-CD14 antibody fluorescence for the presence of P2X7 receptors by flow cytometric analysis.

Oxidized ATP Blocks the ATP-stimulated Release of IL-1β in LPS-primed Monocytes—We then asked the question whether monocytes express functional P2X7 receptors. Therefore, we attempted to determine if the P2X7 receptor antagonist, oxidized ATP, and a P2X7-specific antibody prevents the ATP-stimulated release of IL-1β in LPS-primed human monocytes. Pretreatment with 300 μM oxidized ATP for 30 min completely abolished the ATP-induced release of IL-1β in human monocytes (Fig. 8). Pretreatment with P2X7-specific monoclonal antibody also blocked the ATP-stimulated release of IL-1β to a lesser extent (Fig. 8). The data suggest that the P2X7 receptor is responsible for the ATP effects.

Effect of Signaling Inhibitors on ATP-stimulated Release of IL-1β—We hypothesized that protein kinases may play a significant role in the release of IL-18 and IL-1β from LPS-primed
human monocytes. Initially, we compared the effects of various inhibitors of protein-tyrosine kinases, protein kinase C, and protein kinase A on release of IL-1β from LPS-primed human monocytes (Table I). Whereas platelet-derived growth factor (AG1296) and epidermal growth factor (AG1478) receptor kinase inhibitors and PKA or PKC pathway inhibitors had no effect, tyrphostin AG126, a PTK inhibitor, prevented ATP-induced release of IL-1β from LPS-primed monocytes (~80% inhibition). The effect was dose-dependent and not cytotoxic to the cells in the concentrations used in the experiment. It therefore appears that ATP-stimulated IL-1β release is mediated by an AG126-sensitive tyrosine kinase(s).

**MEK Inhibitors Do Not Block IL-1β Release**—AG126 is a potent inhibitor of PTK and inhibits NO production and ERK activation while protecting mice from LPS-induced septic shock (30, 31). Therefore, we asked whether the AG126 effect on IL-1β release in the presence of LNAME, a nitric-oxide synthase inhibitor, was due to the inhibition of NO production or inhibition of ERK activation.

Using ELISA detection, we first assessed the effects of PTK inhibitors herbinycin A and AG126, mitogen-activated protein kinase kinase inhibitors UO2126 and PD98059, and nitric-oxide synthase inhibitor LNAME on ATP-induced release of IL-1β in LPS-primed monocytes (Fig. 9A). Again, AG126 inhibited IL-1β release in a dose-dependent manner, whereas the other PTK inhibitor herbinycin A and the MEK inhibitors UO2126 and PD98059 did not inhibit ATP induced IL-1β release (Fig. 9A).

To examine whether the AG126 effect was due to the inhibition of NO production, we analyzed ATP-induced IL-1β release in the presence of LNAME, a nitric-oxide synthase inhibitor. Interestingly, instead of the inhibition of release, LNAME enhanced IL-1β release by 2–3-fold. Of note, AG126 totally abolished the LNAME activated release of IL-1β (Fig. 9A).

**Effect of AG126 on ERK Phosphorylation**—We then evaluated whether AG126 inhibits ATP-induced ERK phosphorylation (Fig. 9B). Whereas the classic MEK inhibitors, UO2126 and PD98059, prevented ERK phosphorylation, AG126 did not. In contrast, the MEK inhibitors did not prevent IL-1β and IL-18 release, but AG126 did. Therefore, the results indicate that the activation of mitogen-activated protein kinase pathway is not essential for ATP-induced IL-1β release from LPS-primed monocytes.

**The Mechanism of the AG126 Effect**—It is possible that the tyrosine kinase activity inhibited by AG126 regulates...
expression of ICE (Fig. 10). AG126 inhibited caspase-1 activation and processing
and release of IL-1β and IL-18. LPS-primed monocytes were treated with various inhibitors as described for Fig. 9, and supernatants were subjected to Western blot analysis for mature IL-1β (A) and for IL-18 (B), and p20 caspase-1 (C). The position of both pro- and mature proteins are shown for IL-18. DMSO, Me2SO.

caspase-1 activation. We therefore asked if the inhibition of ATP-stimulated release of cytokines by AG126 is mediated by the inactivation of ICE. To accomplish this we examined the ATP-induced processing and release of IL-1β and IL-18 in the presence of kinase inhibitors. Western blot analysis of the cell supernatants for IL-1β (Fig. 10A) and IL-18 (Fig. 10B) confirmed the ELISA (Fig. 9) results. As it did for IL-1β, AG126 inhibited the processing and release of IL-18. The MEK inhibitors, herbimycin A and the nitric-oxide synthase inhibitor LNAME, did not affect ATP-induced processing and release of the cytokines.

Finally, we examined the effect of AG126 on caspase-1 activation by Western blot analysis. Interestingly, AG126 also inhibited the ICE activation, as the 20-kDa active form of ICE was not detected; whereas, herbimycin A, MEK, and nitric-oxide synthase inhibitors had no effect on ATP-induced activation of ICE (Fig. 10C). Thus the inhibition of IL-1β β release by AG126 correlates with the inhibition of ATP-induced ICE activation, and the processing of ICE appears to be regulated by AG126-sensitive protein-tyrosine kinases.

DISCUSSION

The processing of precursor IL-1β and IL-18 and their secretion into the exterior are key regulatory events in inflammation, yet remain poorly understood. Both proIL-1β and proIL-18 are biologically inactive proteins that require cleavage by caspase-1 for biological activation (8, 9, 32). Both IL-1β and IL-18 share significant structural homology (4, 5), and both lack a leader sequence required for their secretion. Furthermore, although the release of mature IL-1β and IL-18 appears to be linked to the processing of precursor forms by caspase-1, functional ICE has not been detected in monocytes, macrophages, or monocytes cell line THP-1 even when the processing and release of IL-1β was induced by LPS (15, 33).

Previous studies provide strong evidence that extracellular ATP enhances both the magnitude and the velocity of the posttranslational processing and release of proIL-1β from peritoneal murine macrophages and human and microglial cells stimulated with LPS and monocytes (15, 18, 27, 34, 35). This ATP-triggered release of IL-1β occurs via activation of the P2X7 receptor (15, 29). The telescoping of the release and processing events provided by ATP allow a more detailed dissection of the events that regulate the processing and release of IL-1β and IL-18.

In this context, we utilized this unique activity of ATP to compare IL-18 and IL-1β processing and release. We provide evidence that there are marked similarities between IL-1β and IL-18 in this regard, and we used the constitutive presence of proIL-18 to evaluate the role of priming in the release event. First of all, we show that, although IL-18 is present in fresh monocytes, the addition of ATP alone is insufficient to induce IL-18 release. Second, utilizing assays specific for the mature and precursor forms of these cytokines, we demonstrate that the ICE inhibitor, YVAD-cmk, inhibits the ATP-induced maturation of both IL-1β and IL-18 but not their release. Third, we show that exogenous ATP rapidly activates caspase-1 in both primed and unprimed monocytes. Furthermore, this ATP effect is mediated through constitutively present and functional P2X7 receptors. Finally, we show that the ATP-induced activation is, at least in part, dependent upon protein-tyrosine kinase activity, since it is inhibited by the PTK inhibitor AG126 but not by nitric-oxide synthase inhibition or by mitogen-activated protein kinase, PKC, or PAK inhibitors. Thus, we demonstrate that both IL-1β and IL-18 are released in a similar fashion and that LPS priming is necessary for this activation for IL-18 and by implication for IL-18 as well. Furthermore, we provide the first report showing evidence that caspase-1 activation is regulated by a protein-tyrosine kinase activity.

The requirement for LPS priming for the ATP induction of IL-18 was unexpected and is particularly noteworthy. Since LPS priming was necessary for the generation of intracellular IL-1β, we expected that the ATP effect was simply a direct trigger of the ICE activation and protein release pathway. However, the findings with IL-18 provide the first evidence to suggest that ATP alone is not able to trigger either processing or release. This LPS “priming” event was not due to an up-regulation of P2X7 receptors, since our flow cytometry and immunoblot analyses showed constitutive presence of P2X7 receptors with only minimal induction by LPS (Fig. 6).

The role of LPS priming in caspase-1 activation is also worthy of comment. As shown in Fig. 5B, ATP stimulation alone activates caspase-1 without inducing processing of constitutive IL-18 or inducing its export from the cell (Fig. 4B, lanes 4 and 13). However, ATP stimulation after LPS priming induces both caspase-1 activation (Fig. 5B, fourth lane) and IL-18 processing and release (Fig. 4B, lanes 3 and 12). These observations suggest that the ATP effect on the release pathway is distinct from its effect on ICE activation.

In confirmation of prior studies, we found it difficult to document the presence of mature IL-1β or mature IL-18 inside LPS-primed, ATP-stimulated monocytes (Fig. 4, A and B). These data suggest that the processing and release of IL-1β and IL-18 are rapid and probably concurrent events. However, these concurrent events are likely independent of each other, since the caspase-1 inhibitor, YVAD-cmk, did not prevent the release of the larger precursor forms. Nevertheless, these findings do not exclude the possibility that caspase-1 may have a role in release that is separate from its function as a protease. For example, in ICE knockout animals, there is a deficit in release of both the mature and precursor forms of IL-1β (26). This raises the possibility that either caspase-1 itself or caspase-1-affiliated molecules make up part of the protein machinery involved in IL-1β and IL-18 release. Since caspase-1 contains a classic caspase recruitment domain (CARD), one can speculate that CARD-interacting molecules may make up part of this release machinery (37). The protein-protein interactions induced by similar recruitment domains are known to be important in the function of apoptotic caspases (38).

Since it has been reported that freshly isolated human monocytes do not express P2X7 receptors, we were intrigued by the responsiveness of fresh monocytes to ATP in our experiments (20). Flow cytometric and Western blot analysis showed that...
the addition of LPS only marginally induced P2X7 receptor expression. The role of the P2X7 was confirmed by several antibody directed at the P2X7 receptor but not by an isotype
AG126 blocks both ICE processing and IL-1β protective of endotoxin-induced shock in a mouse model (31).
control the processing and release of IL-1β both caspase-1 functions, the identification of this AG126-sen-
tant to note that inhibition of ICE activity by YVAD-cmk does
ice-deficient animals (26). Although IL-18 and IL-1β knockout animals are not protected from septic shock, ICE knockout
AP-activated release of IL-1β
ATP-activated release of IL-1β
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