The Journal of Immunology

RESEARCH ARTICLE | OCTOBER 15 2000

ATP Acts as an Agonist to Promote Stimulus-Induced Secretion of IL-1β and IL-18 in Human Blood FREE

David G. Perregaux; ... et. al

J Immunol (2000) 165 (8): 4615–4623.
https://doi.org/10.4049/jimmunol.165.8.4615

ATP Acts as an Agonist to Promote Stimulus-Induced Secretion of IL-1β and IL-18 in Human Blood

David G. Perregaux, Pat McNiff, Ron Laliberte, Maryrose Conklyn, and Christopher A. Gabel[1]

Cultured monocytes and macrophages stimulated with LPS produce large quantities of proIL-1β, but release little mature cytokine to the medium. The efficiency at which the procytokine is converted to its active 17-kDa species and released extracellularly is enhanced by treating cytokine-producing cells with a secretion stimulus such as ATP or nigericin. To determine whether this need for a secretion stimulus extends to blood, individual donors were bled twice daily for 4 consecutive days, and the collected blood samples were subjected to a two-step IL-1 production assay. LPS-activated blood samples generated cell-free IL-1β, but levels of the extracellular cytokine were greatly increased by subsequent treatment with ATP or nigericin. Specificity and concentration requirements of the nucleotide triphosphate effect suggests a $P2X_7$ receptor involvement. Quantities of IL-1β generated by an individual donor's blood in response to the LPS-only and LPS/ATP stimuli were relatively consistent over the 4-day period. Between donors, consistent differences in cytokine production capacity were observed. Blood samples treated with ATP also demonstrated enhanced IL-18 production, but TNF-α levels decreased. Among leukocytes, monocytes appeared to be the most affected cellular targets of the ATP stimulus. These studies indicate that an exogenous stimulus is required by blood for the efficient production of IL-1β and IL-18, and suggest that circulating blood monocytes constitutively express a $P2X_7$-like receptor. ***The Journal of Immunology*****, 2000, 165: 4615–4623.**

Interleukin 1 has achieved master inflammatory cytokine status because of its ability to initiate a wide variety of proinflammatory activities. For example, IL-1 can promote expression of adhesion molecules on endothelial cells (1), cyclooxygenase 2 by mesangial cells (2), and matrix degrading metalloproteinases by chondrocytes (3). As a result of these many activities, IL-1 is implicated as a contributing factor to several human diseases including rheumatoid arthritis (RA),[2] osteoarthritis, and inflammatory bowel disease (4). However, despite its well accepted role as a potent proinflammatory mediator, levels of IL-1 found in serum and/or synovial fluid from RA patients is very low and difficult to detect (5). Therefore, IL-1 does not appear to circulate in great abundance, but rather elicits its effects locally (6). The importance of maintaining low circulating levels of IL-1 may explain why the natural receptor antagonist of IL-1 is an acute phase protein produced by hepatocytes (7). High circulating levels of the receptor antagonist are likely to function as a buffer to ensure that IL-1, which escapes from a local environment, does not needlessly activate cells and tissues that it may encounter before its clearance.

Activity attributed to IL-1 actually is derived from two separate but related polypeptides, IL-1α and IL-1β. Both of these cytokines are produced as 31-kDa propolypeptides (8, 9). ProIL-1α is fully competent to bind to IL-1 receptors on target cells and to elicit a biologic response (10), but this polypeptide may be cleaved to an equally active 17-kDa mature species by a calpain-type protease (11). In contrast, proIL-1β is incompetent as an agonist of IL-1 receptors (11). Cleavage of this propolypeptide to a 17-kDa species by caspase-1 is required to generate an active signaling molecule (12, 13). Caspase-1 also functions in the activation of the proinflammatory cytokine IL-18 (14). ProIL-1α, proIL-1β, and proIL-18 all are synthesized without signal peptides (9, 15); these signature peptides normally direct a polypeptide that is destined to be secreted to the secretory apparatus of the cell (16). In the absence of the signal peptide, the procytokines accumulate within the cytosolic compartment of activated monocytes/macrophages (17). Release of IL-1 to the medium from monocytes/macrophages that are actively engaged in cytokine synthesis is an inefficient process (18–21). However, the cell-associated molecules can be proteolytically processed and externalized rapidly when the producing cells encounter an extracellular initiator of post-translational processing such as ATP, cytolytic T-cells, bacterial toxins, and potassium ionophores (18, 20–23). These agents ultimately compromise the integrity of the cytokine-producing cell's plasma membrane, suggesting that cellular necrosis and/or apoptosis may accompany IL-1 release (18, 20). However, not all agents that promote cell death cause proteolytic processing of proIL-1β (20), and the possibility exists that IL-1 release in vivo may be achieved in the absence of overt cell lysis.

ATP acts as an agonist of IL-1 post-translational processing by binding to the $P2X_7$ receptor (24–26). Like other members of the P2X family of ligand-gated ion channels, the $P2X_7$ receptor forms a nonselective channel in the presence of ATP (27–30). However, unlike other members of the family, the channel formed by the $P2X_7$ receptor rapidly transitions to a pore-like structure that allows passage of molecules as large as 900 Da (29, 31, 32). Prolonged ligation of the $P2X_7$ receptor ($>$15 min) commits the cell to death (33, 34). By activity, the $P2X_7$ receptor (formerly called P2Z) is restricted to a limited number of cell types including monocytes, macrophages, microglial cells, and some lymphocytes and cancer cells (33, 35). The physiological function of this unusual receptor remains unclear.

Studies that have demonstrated the need for a secondary stimulus to promote efficient IL-1 post-translational processing have employed purified preparations of LPS-activated monocytes, macrophages, and microglial cells (18, 24, 25, 34, 36). One could argue that the isolated

Department of Respiratory, Allergy, Immunology, Inflammation, and Infectious Diseases, Pfizer Central Research, Groton, CT 06340

Received for publication May 17, 2000. Accepted for publication July 24, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Address correspondence and reprint requests to Dr. Christopher A. Gabel, Department of Respiratory, Allergy, Immunology, Inflammation, and Infectious Diseases, Pfizer, Inc., Groton, CT 06340. E-mail address: christopher_a_gabel@groton.pfizer.com

[2] Abbreviations used in this paper: RA, rheumatoid arthritis; SIS, stimulus-induced secretion.

0022-1767/00/$02.00

cells lacked important extracellular components and/or growth factors that were necessary for efficient cytokine processing, and that the requirement for a secretion stimulus (in addition to LPS) was an artifact of the experimental system. However, an in vivo study demonstrated that LPS-activated murine peritoneal macrophages also require a secretion stimulus to achieve maximum IL-1 production capacity (37). To further address this issue, the current study investigates production of IL-1β by human blood. A cohort of 12 individuals was bled twice daily for 4 consecutive days, after which the blood samples were analyzed in a two-step cytokine release assay. Results indicate that LPS-activated human blood samples generate greater levels of IL-1β in the presence of a secretory stimulus such as ATP or nigericin. The magnitude of the increase was consistent over the course of analysis. The ATP stimulus also increased extracellular levels of IL-18, and caused a high percentage of monocytes to demonstrate altered physical properties. These changes in monocytes did not require pretreatment with LPS, suggesting that a high percentage of these cells constitutively express a $P2X_7$-like receptor. Therefore, efficient release of IL-1 from LPS-activated human blood is dependent on extracellular effectors such as ATP. The need for separate stimuli to promote synthesis and release of IL-1β punctuates the importance served by post-translational processing in the regulation of this cytokine's activity.

Materials and Methods

Blood-based cytokine production assay

Blood was collected from normal volunteers and RA patients in heparin-containing vacutainer tubes; these samples could be stored on ice for up to 4 h with no adverse effect on assay performance. A total of 75 μl of blood was placed into an individual well of a 96-well plate and diluted with 75 μl RPMI 1640 medium containing 20 mM HEPES (pH 7.3). The diluted blood samples then were incubated for 2 h in the absence or presence of 100–200 ng/ml LPS (*Escherichia coli* serotype 055:B5; Sigma, St. Louis, MO) at 37°C in a 5% CO_2 environment. After this incubation, ATP was introduced as a secretion stimulus (by addition of the appropriate volume of a solution of 100 mM ATP in 20 mM HEPES (pH 7)) where indicated, and the mixtures were incubated at 37°C for an additional 2 h. The 96-well plates then were centrifuged at 700 $\times$ *g* for 10 min, and the resulting plasma samples were harvested; these samples were stored at −20°C. In some experiments, ADP, UTP, or GTP was substituted for ATP as the secretion stimulus (all were obtained from Sigma). When nigericin was employed as the secretion stimulus, this potassium ionophore (Sigma) was prepared as a 10-mM stock solution in ethanol. Each condition was assayed in a minimum of triplicate wells. None of the agonists or antagonists appeared to cause lysis of RBC as evidenced by the absence of visible hemoglobin within the plasma supernatants.

ELISA measurements

Plasma supernantants were analyzed in the following ELISAs: IL-1β (R&D Systems, Minneapolis, MN), IL-18 (Medical and Biological Laboratories, Nagoya, Japan), and TNF-α (R&D Systems). The assays were performed following the manufacturer's specifications, and absolute cytokine levels were calculated based on comparison to assay performance in the presence of known quantities of recombinant cytokine standards.

Laser flow analysis

Blood was collected in heparin-containing vacutainer tubes. A total of 0.25 ml of each sample was diluted with 0.25 ml of RPMI 1640 medium containing 20 mM HEPES (pH 7.3) in a 1.5 ml Eppendorf tube (Eppendorf Scientific, Westbury, NY). The diluted samples were incubated for 2 h in the absence or presence of 100 ng/ml LPS at 37°C. ATP then was introduced (by addition of 0.03 ml of a 100 mM stock solution) where indicated, and the samples were incubated for an additional 2 h at 37°C. These incubation mixtures then were analyzed on a Cell Dyne (Abbott Laboratories, Abbott Park, IL; model 3500) for leukocyte content.

FACS analysis

Blood samples (collected in heparin) were diluted with an equal volume of RPMI 1640 medium containing 20 mM HEPES (pH 7.3) in polypropylene tubes. Where indicated, ATP was added to achieve a final concentration of 6 mM, and the samples were incubated at 37°C for 2 h. The 0.1 ml blood samples then were centrifuged, the plasma supernatants were discarded, and the cells were suspended in 1 ml of PBS and again were subjected to centrifugation. The resulting cell pellets were suspended in 10 μl of heat-aggregated human IgG and incubated at 4°C for 10 min, after which 10 μl FITC-conjugated anti-CD33 and 10 μl PE-conjugated anti-CD14 (both mAbs were obtained from PharMingen, San Diego, CA) were added and the samples were incubated for an additional 30 min at 4°C. At this point, 1 ml of PBS containing 2% FCS and 0.2% sodium azide (PBS^+) was added to each sample and the cells were collected by centrifugation. These cell pellets were suspended in 1 ml of FACS lysing solution (Becton Dickinson, Franklin Lakes, NJ) and the samples were incubated for 10 min at room temperature to lyse RBC. Intact leukocytes then were collected by centrifugation, washed three times with PBS^+ by repeated centrifugation, and resuspended in 0.5 ml of PBS^+. These samples were analyzed by FACScan; a total of 20,000 ungated events were collected per analysis.

Results

IL-1 release from LPS-activated blood occurs via a stimulus-coupled response

Freshly isolated human monocytes maintained in culture produce large quantities of proIL-1β in response to LPS activation, but release little of the cytokine product to the medium as the mature polypeptide species (21, 24, 26, 38, 39). However, the subsequent addition of ATP to the LPS-activated cells causes a high percentage of the cytokine to be externalized as the mature polypeptide species (21, 24, 26, 39). Monocytes are considered to be the major cell-type in blood responsible for IL-1 production (40). To determine whether the blood environment alters cytokine production requirements, individual blood samples from 12 normal volunteers were stimulated with LPS for 2 h and then treated with or without ATP for an additional 2 h. IL-1β released to the medium subsequently was determined by ELISA. In the absence of LPS and ATP, no significant IL-1 was produced by any of the blood samples (Fig. 1). Addition of LPS in the absence of ATP caused production of measurable quantities of IL-1 in 10 of the 12 blood samples (Fig. 1). Addition of ATP to the LPS-activated samples greatly enhanced the quantity of IL-1 generated by all 12 blood samples (Fig. 1). The relative increase in cytokine levels produced by the LPS-treated samples in the presence and absence of ATP, referred to as the stimulus-induced secretion (SIS) ratio, ranged from a low of 3.9 for subject III to an incalculable value for subjects X and XI (Fig. 1). In the absence of prior LPS stimulation, ATP did not cause production of IL-1 by blood samples (see below).

The stimulus-coupled response demonstrates a concentration dependence for ATP (Fig. 2). LPS-activated blood treated with concentrations of ATP $\leq$0.5 mM produced quantities of IL-1 comparable to those generated in the absence of the nucleotide triphosphate (Fig. 2). In contrast, concentrations of ATP $\geq$1 mM increased levels of extracellular cytokine well above those observed in the absence of the nucleotide triphosphate; maximum production was observed at 6 mM ATP, the highest concentration tested (Fig. 2). In contrast to ATP, the nucleotide triphosphates UTP and GTP and the nucleotide diphosphate ADP were inactive as secretion stimuli (Fig. 2 inset).

The K^+ ionophore nigericin promotes IL-1β post-translational processing by LPS-activated monocytes in culture (20). Likewise, nigericin served as a secretion stimulus in blood. LPS-activated blood samples incubated in the absence of the ionophore generated minimal levels of extracellular cytokine (Fig. 3). Addition of nigericin to the LPS-activated samples stimulated cytokine release; a final concentration of 100 μM nigericin appeared to be optimal (Fig. 3). Quantities of IL-1β produced in the presence of nigericin were comparable to those produced by 6 mM ATP.

The standard assay developed for measuring IL-1 release involved diluting the blood samples 2-fold with RPMI 1640 medium. In preliminary experiments, blood samples were employed

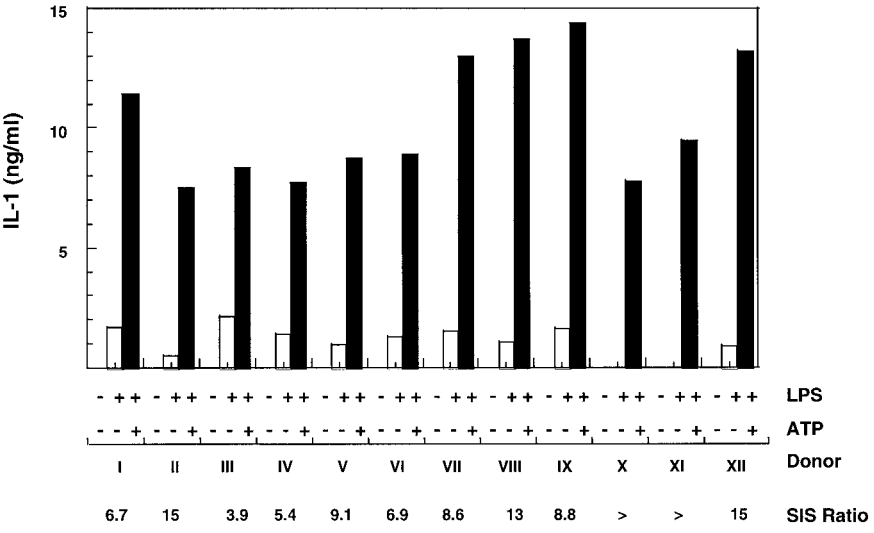

FIGURE 1. Extracellular ATP enhances quantities of IL-1β produced by LPS-activated human blood. Samples of blood from normal volunteers (donors I-XII) were incubated for 2 h in the absence (−) or presence (+) of 200 ng/ml LPS followed by an additional 2-hr incubation the absence or presence of 6 mM ATP. Plasma supernatants then were harvested and assayed for IL-1β content by ELISA. The quantity of IL-1β (ng/ml) released is indicated as a function of treatment for each individual donor. Each data point is the mean of quadruplicate determinations. The SIS ratio is indicated for each donor's blood; this ratio represents the amount of IL-1β produced by the combination of LPS/ATP divided by the amount generated by LPS only. The larger the SIS ratio, the greater the dependence on ATP for cytokine externalization.

without dilution and identical results were obtained with respect to the need for a secretion stimulus (data not shown). However, assay performance was found to be more reproducible when the media dilution step was included.

Blood IL-1 production is constant and not subject to diurnal variance

To determine whether the observed characteristics of the IL-1β production assay were consistently demonstrated, the same 12 subjects who donated blood for the assay shown in Fig. 1 were bled twice daily for 4 consecutive days. After each bleed, the blood samples were subjected to the two-step IL-1 release assay. Although differences were observed in the total quantity of IL-1β released in response to the +LPS/+ATP combination between individual subjects, the quantities produced by any one individual were remarkably consistent throughout the 78-h time frame encompassed by the eight assays (Table I and Fig. 4*A*). For example, subjects XII and VIII generated higher amounts of IL-1β in response to LPS/ATP on average than did the other 10 subjects. Total IL-1β produced over the 4-day period for these two individuals ranged from a low of 13 ng/ml to a high of 19 ng/ml (Table I). In contrast, subjects II and V consistently generated less IL-1β; in response to LPS/ATP, blood samples from these subjects generated cytokine levels ranging between 5.8 and 8.9 ng/ml (Table I).

Remarkably, the SIS ratio also demonstrated consistency over the 4-day period. For example, the response ratio observed at each bleed time for subject II ranged between a low value of 15 and a high value of 56; the mean and SD over the eight bleeds was 35 ± 13. In contrast, the observed SIS ratios for subject III were consistently lower and ranged between 4 and 7.6 (Fig. 4*B*); the mean and SD over the eight bleeds for this individual were 5.5 ± 1.2. The SIS response ratio for subject VI was intermediate to that observed for subjects II and III, and ranged between 6.8 and 16 (Table I and Fig. 4*B*); the mean and SD over the eight bleeds were 11 ± 3.9. Therefore, quantities of IL-1 released in response to the LPS-only and the LPS/ATP stimuli are consistent traits of an individual's blood.

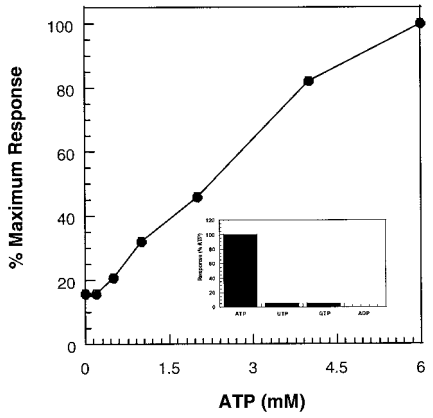

FIGURE 2. ATP enhances blood IL-1β production via a concentration-dependent and selective mechanism. Blood from a single volunteer was activated with 100 ng/ml LPS for 2 h, after which the indicated concentration of ATP was added and the samples were incubated for an additional 4 h. Plasma supernatants then were harvested and assayed for IL-1β by ELISA. The quantity of IL-1β (expressed as % of the maximum response observed at 6 mM ATP) is indicated as a function of ATP concentration. Each data point is the mean of quadruplicate determinations. The inset compares the ability of ATP, UTP, GTP, and ADP to promote cytokine release from LPS-activated blood.

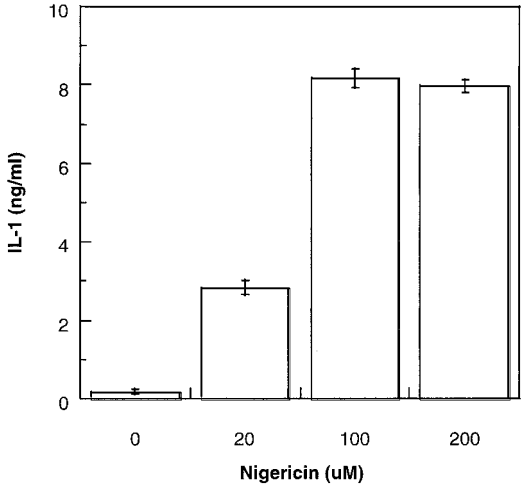

FIGURE 3. Nigericin induces IL-1β externalization from LPS-activated human blood. Blood from a single individual was activated for 2 h in the presence of 100 ng/ml LPS and then incubated with the indicated concentration of nigericin (containing a final concentration of 1% vehicle (ethanol) in all wells) for an additional 2 h. Plasma supernatants then were harvested and assayed for IL-1β content by ELISA. The quantity of IL-1β (ng/ml) recovered is indicated as a function of nigericin concentration. Each bar is the mean (± SD) of quadruplicate determinations.

Table I. *Donor dependence of the blood-based IL-1β production assay*[a]

		IL-1β (ng/ml)							
		Day 1		Day 2		Day 3		Day 4	
Donor	Treatment	8:30 a.m.	2:30 p.m.	8:30 a.m.	2:30 p.m.	8:30 a.m.	2:30 p.m.	8:30 a.m.	2:30 p.m.
I (m)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	1.7	1.2	0.72	1.0	0.88	0.94	2	0.72
	+LPS/+ATP	11	9.0	8.2	11	8.1	7.5	10	11
	SIS Ratio	6.7	7.5	11	11	9.2	8	5.2	15
II (f)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	0.5	0.26	0.2	0.16	0.2	0.22	0.32	0.16
	+LPS/+ATP	7.5	7.0	7.1	7.0	7.3	8.2	7.8	8.9
	SIS Ratio	15	27	36	44	37	37	24	56
III (f)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	2.1	1.8	1.2	1.6	1.1	1.8	1.2	1.3
	+LPS/+ATP	8.3	7.5	7.1	8.6	7.0	8.7	6.4	9.9
	SIS Ratio	4	4.2	5.9	5.4	6.4	4.8	5.3	7.6
IV (m)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	1.4	0.32	0.48	0.44	0.4	0.36	0.5	0.12
	+LPS/+ATP	7.7	9.3	7.5	10	11	9.4	10	11
	SIS Ratio	5.5	29	16	23	27	26	21	89
V (m)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	0.96	1.2	0.76	1.1	0.88	0.72	0.8	0.68
	+LPS/+ATP	8.7	6.6	7.7	7.0	6.6	7.1	5.8	7.6
	SIS Ratio	9.1	5.5	10	6.4	7.5	9.9	7.3	11
VI (f)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	1.3	0.98	0.48	1	1.1	1.1	0.58	0.68
	+LPS/+ATP	8.9	8.4	7.0	11	7.6	9.2	8.7	11
	SIS Ratio	6.8	8.6	15	11	6.9	8.4	15	16
VII (m)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	1.5	2.1	1.1	1.3	1.5	2.3	1.3	1.3
	+LPS/+ATP	13	16	18	12	9.8	19	14	13
	SIS Ratio	8.7	7.6	16	9.2	6.5	8.3	11	10
VIII (m)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	1.1	1.7	1.8	1.6	1.4	3.3	1.2	1.2
	+LPS/+ATP	14	17	18	13	13	19	14	16
	SIS Ratio	13	10	10	8.1	9.3	5.8	12	13
IX (m)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	1.6	0.6	0.3	0.24	0	0.5	0.7	0.12
	+LPS/+ATP	14	14	15	9.8	11	17	15	17
	SIS Ratio	8.8	23	50	41	>	34	21	140
X (f)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	0	0.44	0	0	0	0.48	0	0
	+LPS/+ATP	7.8	9.4	9.3	6.5	8.1	10	6.4	7.7
	SIS Ratio	>	21	>	>	>	21	>	>
XI (m)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	0	0.24	0	0.2	0.16	0	0.26	0.36
	+LPS/+ATP	9.4	12	14	9.8	10	15	14	13
	SIS Ratio	>	50	>	49	63	>	54	36
XII (m)	−LPS/−ATP	0	0	0	0	0	0	0	0
	+LPS/−ATP	0.88	1.4	1.5	0.86	0.92	1.4	1.0	0.36
	+LPS/+ATP	13	16	19	13	13	19	13	13
	SIS Ratio	15	11	13	15	14	14	13	36

[a] Normal volunteers were bled twice daily (8:30 a.m. and 2:30 p.m.) for 4 consecutive days. Each bleed immediately was subjected to the two-step assay protocol with the indicated effector combination (Treatment). IL-1β released to the supernatants subsequently was determined by ELISA and is indicated (ng/ml) for each treatment and time. The stimulus-induced secretion (SIS) ratio also is indicated; this ratio is obtained by dividing the quantity of IL-1 produced in the presence of the +LPS/+ATP effector combination by the level generated with the +LPS/−ATP combination. Individual donors are indicated. m and f, Male and female, respectively. >, A SIS ratio could not be calculated due to a lack of secretion in the presence of LPS only.

Blood samples from RA patients also require a secretion stimulus for efficient IL-1β release

Literature reports suggest that blood-borne cells obtained from RA patients may be in an activated state and circulate with IL-1 on board (41, 42). To explore the possibility that blood samples obtained from RA patients may perform differently in the IL-1 production assay, several samples of blood from affected individuals were analyzed; no consideration of current therapy was taken into account in the selection of these individuals. Preliminary experiments indicated that blood samples derived from normal volunteers could sit on ice for up to 4 h with no change in their cytokine production properties (data not shown). RA patient blood samples obtained at a local rheumatologist's office were transported to the lab on ice and then subjected to the two-step IL-1β production assay. In the absence of LPS stimulation,

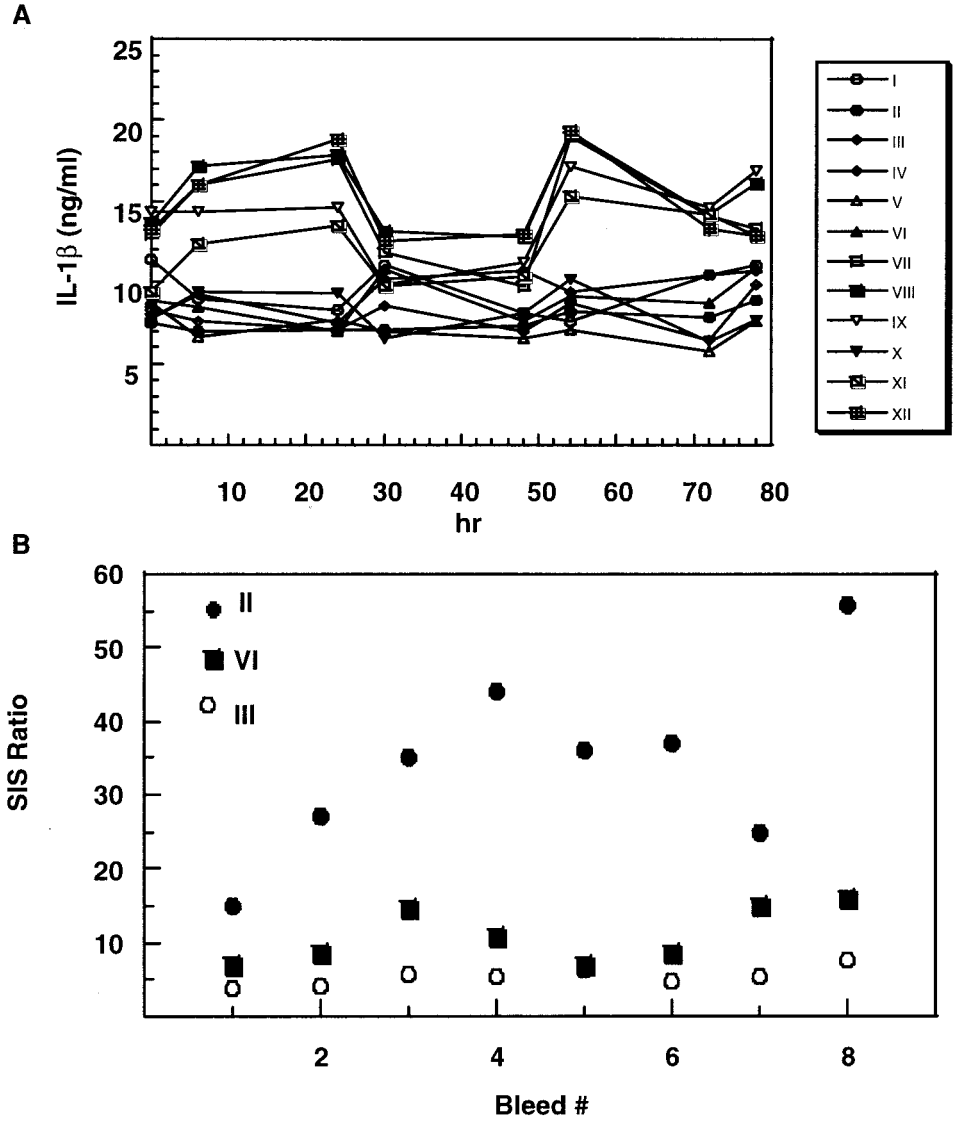

FIGURE 4. Performance of the blood-based IL-1β production assay is donor dependent. Twelve donors were bled twice daily for 4 consecutive days. Each bleed was immediately subjected to the two-step analysis and the quantity of IL-1β released to the plasma supernatants was determined by ELISA. *A*, Total quantities of IL-1β produced in response to the combination of LPS and ATP are indicated as a function of bleed time (in hours) for each individual donor. *B*, The SIS ratio observed as a function of the bleed number is indicated for three of the donors.

no significant IL-1β was detected in three separate samples (Table II); this was true for blood samples treated with (−LPS/+ATP) or without (−LPS/−ATP) ATP. Addition of LPS (+LPS/−ATP) led to generation of IL-1β by all three blood samples; IL-1β levels ranged between 0.023 to 0.2 ng/ml (Table II). Treatment of the same blood samples with the combination of LPS and ATP (+LPS/+ATP) generated quantities of IL-1β ranging from 1.7 to 10.4 ng/ml (Table II). The lower quantities of IL-1β produced by RA patient A correlated with a reduced number of blood monocytes (Table II). Therefore, performance of RA patient blood samples in the IL-1β production assay is similar to that observed with samples obtained from normal healthy subjects.

IL-18 release is enhanced by ATP treatment

IL-18, like IL-1β, is produced as a leaderless propolypeptide that requires cleavage by caspase-1 to generate an active mature cytokine (14). To determine whether ATP enhanced IL-18 production, blood samples were subjected to the two-step activation protocol and cytokine levels were assessed by ELISA. IL-1β production by these blood samples behaved as expected. In the absence of an activator (−LPS/−ATP) no IL-1β was detected, and treatment with ATP alone (−LPS/+ATP) did not enhance cytokine production (Table III). In contrast, blood samples treated with LPS individually (+LPS/−ATP) or in combination with ATP (+LPS/+ATP) generated 1.9 and 16.1 ng/ml, respectively, of IL-1 (Table III). In these same blood samples, IL-18 production demonstrated a different response pattern. In the absence of a stimulus (−LPS/−ATP), 0.04 ng/ml IL-18 were detected. Treatment with LPS only (+LPS/−ATP) did not increase levels of IL-18 significantly, but samples treated with ATP only (−LPS/+ATP) yielded 0.29 ng/ml, or a 7-fold increase above the inactivated comparator. The combination of LPS and ATP (+LPS/+ATP) promoted a slightly higher yield of IL-18 corresponding to 0.38 ng/ml (Table III).

In contrast to IL-1β and IL-18, TNF-α is secreted from cells via a process involving the normal cellular secretory apparatus consisting of the endoplasmic reticulum and Golgi apparatus (43). To assess whether this type of secretory product was affected by ATP, the same blood samples used above were analyzed for their content of TNF-α. As expected, TNF-α was produced in samples stimulated with LPS but not in samples stimulated with ATP only (Table III). The combination of LPS and ATP decreased TNF-α production relative to blood samples stimulated with LPS only (Table III).

ATP alters specific leukocyte populations

ATP binding to the $P2X_7$ receptor can promote cell volume changes and death if the receptor remains ligated for >15 min (33,

Table II. *Blood samples from RA patients demonstrate stimulus-coupled IL-1 release*[a]

RA Patient	IL-1β (ng/ml)				Monocytes/ml
	−LPS/−ATP	+LPS/−ATP	−LPS/+ATP	+LPS/+ATP	
A	0	.023	0	1.7	1.4×10^5
B	0	.06	0	8.7	5.2×10^5
C	0	0.2	0	10.4	5.5×10^5

[a] Blood samples from three individual RA patients were subjected to the two-step assay protocol with the indicated treatment, after which the blood cells were removed by centrifugation and plasma supernatants were harvested. IL-1β levels within the supernatants were determined by ELISA. Samples of the three blood specimens (prior to any treatment) also were analyzed by laser flow analysis to determine the number of monocytes present.

34). To identify leukocyte populations responsive to ATP, blood samples from three separate donors were subjected to the two-step IL-1β production assay after which they were characterized by Cell Dyne laser flow analysis; this instrument distinguishes individual leukocytes based on their size and granularity (44). For donor no. 1, the blood sample treated with ATP (−LPS/+ATP) yielded 12% fewer total leukocytes than the sample exposed to no stimulus (−LPS/−ATP; Table IV). Neutrophil and eosinophil numbers were essentially unchanged between these two blood samples, but lymphocyte and monocyte numbers decreased by 39 and 93%, respectively (Table IV). Basophil numbers also decreased after ATP treatment; however, based on their limited number it is not clear that this change reflects an actual decline in the number of basophils or of a contaminating cell-type. Treatment with LPS alone (+LPS/−ATP) reduced total leukocyte counts by 10% vs the untreated blood sample, with a reduction in the number of monocytes accounting for the bulk of this change; loss of monocytes may occur as a result of the activated cells adhering to the plastic culture tubes and/or changing shape such that they no longer appear in the gated window. Relative to the LPS-only treatment, blood treated with the combination of LPS and ATP (+LPS/+ATP) contained a reduced number of total leukocytes, and this loss again reflected reductions in the monocyte and lymphocyte populations (Table IV). Similar trends were observed with blood obtained from two other donors (Table IV). ATP treatment of each of the three blood samples, either alone or in combination with LPS, reduced lymphocyte and monocyte numbers without significantly affecting the numbers of neutrophils and eosinophils (Table IV). This effect of ATP was not shared with UTP. Addition of 6 mM ATP reduced the percentage of monocytes and lymphocytes within non-LPS treated blood samples (Table V). In contrast, the same concentration of UTP produced no change in leukocyte composition (Table V).

To further establish that blood-borne monocytes were ATP-responsive, FACS analysis was performed. Human blood samples incubated with or without ATP were treated with specific fluorescently tagged Abs to identify monocytes (anti-CD14 and anti-CD33; 45). Within non-ATP-treated samples, 5.1% of leukocytes stained positive with anti-CD33 and demonstrated the light scatter profile expected of monocytes (Fig. 5). In contrast, the percentage of leukocytes that stained positive for CD-33 following ATP treatment was reduced to 2.9%, and the positive cells were distributed between two distinct populations. Some of the CD-33-positive cells persisted within the original monocyte position, but a new population possessing reduced forward and side scatter signals was apparent within the ATP treated samples (Fig. 5). Thus, a volume and/or shape change occurs to a high percentage of monocytes following ATP treatment, resulting in their failure to gate in the expected region of monocytes. In the same experiment, monocytes were also traced using PE-labeled anti-CD14. Following ATP treatment, a lower percentage of CD-14-positive cells was detected, and the fluorescence intensity of the positive cells was reduced. This reduction in fluorescence intensity suggests that the CD-14 Ag is cleaved and released post-ATP activation. It should be noted that blood samples subjected to the FACS analysis were not pretreated with LPS.

Table III. *IL-1β, IL-18, and TNFα demonstrate different production requirements*[a]

Treatment	Cytokine (ng/ml)		
	IL-1β	IL-18	TNFα
−LPS/−ATP	0	0.04	0
+LPS/−ATP	1.9	0.05	8.0
−LPS/+ATP	0	0.29	0
+LPS/+ATP	16.1	0.38	2.3

[a] Blood obtained from an individual normal volunteer was subjected to the two-step assay protocol with the indicated treatment after which the blood cells were removed by centrifugation and plasma supernatants were harvested. Cytokine levels were determined by ELISA. This experiment was repeated twice (separate donors) with comparable results.

Discussion

LPS promotes synthesis of a variety of cytokines when added to blood (46). Within this complex mixture of cell-types, the monocyte is thought to be the cell of origin for most IL-1 production (39). The ultimate amount of IL-1 generated in response to LPS is governed by a number of transcriptional and translational processes (47, 48). Though less well understood, post-translational processing also represents an important determinant of the amount of active IL-1 generated in response to LPS activation. Studies with isolated monocytes and macrophages have demonstrated that IL-1 release in the absence of a secretion stimulus is an inefficient process. For example, LPS-stimulated mouse peritoneal macrophages produce large quantities of proIL-1β, but release minimal quantities of the newly synthesized cytokine product to the medium (18, 20). In the absence of its release, the cell-associated cytokine is degraded through turnover (19, 20). Likewise, human monocytes aged in culture generate large quantities of proIL-1β when stimulated with LPS, but these cells inefficiently release the newly synthesized polypeptides to the medium (21, 24, 26, 38). Freshly isolated human monocytes release a higher percentage of their IL-1β following LPS challenge than do cells that are aged in culture before LPS activation (39, 49). The reason for this difference in secretory activity between monocyte populations is unclear; serum factors and cytokines are known to affect the ability of cultured monocytes to process proIL-1β, suggesting that the cell's secretory capacity is dynamic and subject to regulation (39).

Stimulation of human blood with LPS caused IL-1 immunoreactivity to be released extracellularly. However, like the behavior of isolated human monocytes, the quantity of IL-1 released in response to LPS represented only a fraction of the total cytokine

Table IV. *ATP treatment differentially affects leukocyte populations*[a]

	Cell Count (cells/μl within assay mixture)			
	−LPS/−ATP	−LPS/+ATP	+LPS/−ATP	+LPS/+ATP
Donor no. 1				
Neutrophils	1930	1970	1850	1930
Lymphocytes	457	282	388	282
Monocytes	187	14	80	8
Eosinophils	68	65	59	57
Basophils	12	4	12	8
Total	2650	2330	2385	2270
Donor no. 2				
Neutrophils	1020	936	957	997
Lymphocytes	914	300	867	368
Monocytes	226	13	53	5
Eosinophils	37	32	35	32
Basophils	24	9	15	9
Total	2220	1290	1930	1410
Donor no. 3				
Neutrophils	2250	2260	2230	2210
Lymphocytes	1480	598	1470	639
Monocytes	296	13	179	10
Eosinophils	45	52	47	32
Basophils	41	8	36	13
Total	4110	2930	3960	2900

[a] Blood samples obtained from three separate normal volunteers were treated with the indicated effectors in the two-step assay protocol, and then leukocyte populations were analyzed by laser flow analysis. Each value listed is an average of duplicate determinations. To ensure that the 4-h incubation itself did not distort the leukocyte counts, a sample of blood obtained from donor no. 2 was analyzed immediately after collecting (diluted 2-fold with medium). This sample yielded 1100 neutrophils, 1040 lymphocytes, 290 monocytes, 33 eosinophils, and 18 basophils; these values are comparable to those obtained after 4 h of incubation in the absence of an effector (−LPS/−ATP).

generated in response to the inflammatory stimulus. The subsequent addition of an exogenous secretion stimulus consisting of ATP or nigericin increased extracellular IL-1β levels dramatically. Similarly, ATP treatment enhanced release of IL-18 from blood, but the nucleotide triphosphate did not elevate extracellular levels of TNF-α. Synthesis of IL-18 was not as dependent on LPS as was IL-1β. This observation is consistent with findings of a previous study that found IL-18 to be constitutively produced by peripheral blood monocytes, whereas IL-1β was synthesized only after LPS activation (50). Despite differences in their expression requirements, both cytokine products require a secretion stimulus such as ATP to achieve efficient release. ATP promotes release of mature IL-1β from monocytes and macrophages (18, 20, 24, 26, 39), and we assume that the cytokine products released in the blood assay also correspond to mature polypeptides; the ELISA kit employed recognizes mature IL-1β and, to a lesser degree, the prospecies (51). In contrast to its ability to enhance production of IL-1β and IL-18, ATP decreased production of TNF-α; death of the cytokine producing monocytes may account for this reduction.

A number of studies with isolated human monocytes provide compelling evidence that ligation of the $P2X_7$ receptor can activate proIL-1β post-translational processing (24–26), and a human monocyte cDNA library was employed as a source for cloning the human receptor (28). Agonist requirements of the blood response are consistent with a $P2X_7$ receptor involvement. ATP, but not UTP, GTP, or ADP, stimulated IL-1 release, and the concentrations of ATP required to promote the response ($\geq$1 mM) are in line with requirements of the $P2X_7$ receptor (28, 29). The effectiveness of ATP is somewhat surprising based on conclusions of several previous studies noting that only a small percentage of isolated blood monocytes express $P2X_7$ receptor activity (52, 53). These studies demonstrated an inability of ATP to induce accumulation of a fluorescent dye (52) or to promote sustained depolarization (53) of freshly isolated monocytes. However, following in vitro differentiation of the monocytes to macrophages a higher percentage of cells demonstrated these $P2X_7$ receptor-associated activities (52, 53). In contrast, our findings demonstrate that ATP is an effective stimulus of IL-1 release in blood and this effect appears to be a monocyte $P2X_7$-mediated response. To explain this discrepancy, we considered the possibility that only a small percentage of monocytes actually expressed the $P2X_7$ receptor, and this subpopulation responded to ATP and released its cytokine load. This

Table V. *ATP, but not UTP, alters leukocyte composition*[a]

	No Effector		+6 mM ATP		+6 mM UTP	
Cell Type	Cell No.	%	Cell No.	%	Cell No.	%
Neutrophils	1090	51	1030	77	814	49
Lymphocytes	876	41	255	19	669	41
Monocytes	150	7	11	0.8	130	7.9
Eosinophils	22	1	32	2.4	18	1.1
Basophils	18	0.8	5	3.8	16	1

[a] Blood from a normal volunteer was treated with the indicated effector for 2 h, and then leukocyte populations were analyzed by laser flow analysis. Both the total number of indicated cells (cell no.) and the percentage of total cells represented by the indicated cell type (%) are indicated. Each value is an average of duplicate determinations.

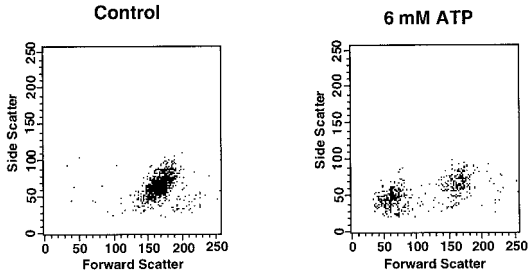

FIGURE 5. Blood-borne CD33-positive monocytes demonstrate an altered light scatter profile after ATP treatment. Samples of blood were incubated in the absence (control) or presence of 6 mM ATP for 2 h and then exposed to an FITC-conjugated anti-CD33 mAb. The blood samples subsequently were analyzed by FACS. The light scatter profiles of the CD33-positive cells are indicated for the two samples.

possibility seems unlikely because the total quantities of IL-1 produced in response to nigericin and ATP were comparable, and nigericin is expected to act independently of the $P2X_7$ receptor. Moreover, a high percentage of monocytes (>90%) in blood samples treated with ATP responded and changed their light scatter properties, suggesting that a majority of blood-borne monocytes possess the $P2X_7$ receptor. The blood-borne monocyte responded to ATP even without prior LPS activation, suggesting that unstimulated monocytes express the ATP receptor. Differences in protocols between the current study and the two previous studies may account for the dissimilar findings. In the IL-1 production assay, blood cells are treated with ATP for longer times than employed in the earlier studies. In addition, plasma components were present in the blood-based IL-1 assay and these components were not present in the previous cultured monocyte experiments. External factors are known to influence $P2X_7$ receptor activity; for example, THP-1 cells do not constitutively express the $P2X_7$ receptor, but after treatment with a combination of LPS and IFN-γ, both $P2X_7$ receptor message and functional protein are expressed (54).

Alternatively, perhaps the monocyte $P2X_7$ receptor demonstrates only a subset of activities that have been attributed to this receptor in other cell types. For example, BW5147 mouse lymphoma cells express the message for the $P2X_7$ receptor and respond to high ATP concentrations by activating a calcium conductance (55); this conductance was inhibited by KN-62, an antagonist of the $P2X_7$ receptor. However, BW5147 cells do not accumulate ethidium bromide in response to ATP, suggesting that their receptor does not form a pore-like structure. Interestingly, a recent report presented evidence suggesting that the $P2X_7$ receptor and the pore represent distinct functional units (56). If true, then perhaps monocytes lack the essential pore component and/or the $P2X_7$ receptor fails to recruit it appropriately.

Laser flow analysis of ATP-treated blood samples indicated that some lymphocytes responded to the nucleotide triphosphate in addition to monocytes; the most abundant white cell population, neutrophils, in contrast, showed no response to the ATP stimulus. To our knowledge, neutrophils and eosinophils have not been reported to express this receptor. At present, it is not clear whether the responding lymphocytes represent a previously characterized subset of these cells. Isolated murine (57) and human (58–60) lymphocytes are reported to demonstrate $P2X_7$ receptor activity, but properties of the lymphocyte-associated pore appear distinct from those typically attributed to the $P2X_7$ receptor (61). Additional work is needed to characterize the T cell response. Therefore, leukocyte populations that we observed to respond in ATP-treated blood samples correspond to cell types known from in vitro studies to express $P2X_7$ receptor activity.

The total amount of IL-1β produced by an individual donor's blood in response to the combination of LPS and ATP was consistent throughout eight different assays over a 4-day period. This indicates a lack of a significant diurnal variation in cytokine production capacity. Surprisingly, a significant donor dependence was observed with respect to the SIS ratio. This ratio reflects the amount of IL-1 released in response to the combination of LPS/ATP relative to amount released with LPS only. Over the 4-day period, blood cells from each individual released a remarkably consistent SIS ratio. This consistency suggests that the inherent ability of a monocyte to release IL-1 in response to LPS is influenced by genetic factors and/or physiological state. Both the total amount of IL-1β released in response to LPS and ATP and the SIS ratio appear to be gender-independent traits. Peripheral blood mononuclear cell preparations isolated from men and women previously were shown to differ with respect to the basal unstimulated secretion of IL-1β, but not with respect to the LPS-inducible component (62). The efficiency at which purified LPS-activated monocytes release IL-1β also is known to be affected by the absolute LPS concentration (63); in the current study, a limited range of LPS concentrations was employed (100–200 ng/ml), and we have not investigated whether changes in LPS concentration affect the SIS ratio.

Blood samples obtained from RA patients behaved comparably to samples obtained from normal volunteers with respect to the two step IL-1β production assay. These samples required both LPS and ATP for maximal production of cell-free IL-1β. In the presence of only ATP, quantities of IL-1β detected extracellularly were minimal and not different from untreated blood samples. Previous studies suggested that circulating blood cells in RA patients are in an activated state and express cell-associated IL-1 (41, 42). Because ATP alone did not promote release of IL-1 from the RA patient blood samples, we conclude that the level of cell-associated cytokine was minimal relative to their LPS-induced production capacity, or that the cells producing IL-1 did not express the $P2X_7$ receptor.

Demonstration that blood-borne monocytes require an exogenous stimulus to efficiently generate cell-dissociated IL-1β and IL-18 provides additional evidence that the process of stimulus-induced secretion is important and physiologically relevant. The requirement for a separate stimulus to promote post-translational processing may provide a final checkpoint to ensure that a monocyte and/or macrophage does not inadvertently release multipotential inflammatory mediators such as IL-1 and IL-18 in the absence of a genuine need. Identification of this post-translational processing checkpoint offers the possibility of identifying novel therapeutic strategies aimed at controlling inflammatory mediators whose export is dependent on this unusual cellular process.

References

1. Bevilacqua, M. P., S. Stengelin, M. A. Gimbrone, Jr., and B. Seed. 1989. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science 243:1160.*
2. Guan, Z., S. Y. Buckman, B. W. Miller, L. D. Springer, and A. R. Morrison. 1998. Interleukin 1-β-induced cyclooxygenase-2 expression requires activation of both c-Jun NH_2-terminal kinase and p38 MAPK signal pathways in rat renal mesangial cells. *J. Biol. Chem. 273:28670.*
3. Flannery, C. R., C. B. Little, B. Caterson, and C. E. Hughes. 1999. Effects of culture conditions and exposure to catobolic stimluators (IL-1 and retinoic acid) on the expression of matrix metalloproteinases (MMPs) and disintegrin metalloproteinases (ADAMs) by articular cartilage chondrocytes. *Matrix Biol. 18:225.*
4. Dinarello, C. A. 1996. Biologic basis for interleukin-1 in disease. *Blood 87:2095.*
5. Holt, I., R. G. Cooper, J. Denton, A. Meager, and S. J. Hopkins. 1992. Cytokine inter-relationships and their association with disease activity in arthritis. *Br. J. Rheum. 31:725.*
6. Arend, W. P., and J.-M. Dayer. 1995. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor α in rheumatoid arthritis. *Arthritis Rheum. 38:151.*

7. Gabay, C., M. F. Smith, Jr., D. Eidlen, and W. P. Arend. 1997. Interleukin 1 receptor antagonist (IL-1RA) is an acute-phase protein. *J. Clin. Invest. 99:2930.*
8. Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Natl. Acad. Sci. USA 81:7907.*
9. March, C. J., B. Mosley, A. Larsen, D. P. Cerretti, G. Braedt, V. Price, S. Gillis, C. S. Henney, S. R. Kronheim, K. Grabstein, P. J. Conlon, T. P. Hopp, and D. Cosman. 1985. Cloning sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature 315:641.*
10. Mosley, B., D. L. Urdal, K. S. Prickett, A. Larsen, D. Cosman, P. J. Conlon, S. Gillis, and S. K. Dower. 1987. The interleukin-1 receptor binds the human interleukin-1α precursor but not the interleukin-1β precursor. *J. Biol. Chem. 262: 2941.*
11. Carruth, L. M., S. Demczuk, and S. B. Mizel. 1991. Involvement of a calpain-like protease in the processing of the murine interleukin 1α precursor. *J. Biol. Chem. 266:12162.*
12. Cerretti, D. P., C. J. Kozlosky, B. Mosley, N. Nelson, K. Van Ness, T. A. Greenstreet, C. J. March, S. R. Kronheim, T. Druck, L. A. Cannizzaro, et al. 1992. Molecular cloning of the interleukin-1β converting enzyme. *Science 256:97.*
13. Thornberry, N. A., H. G. Bull, J. R. Calaycay, K. T. Chapman, A. D. Howard, M. J. Kostura, D. K. Miller, S. M. Molineaux, J. R. Weidner, J. Aunins, et al. 1992. A novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes. *Nature 356:768.*
14. Gu, Y., K. Kuida, H. Tsutsui, G. Ku, K. Hsiao, M. A. Fleming, N. Hayashi, K. Higashino, H. Okamura, K. Nakanishi, et al. 1997. Activation of interferon-γ inducing factor mediated by interleukin-1β converting enzyme. *Science 275:206.*
15. Ushio, S., M. Namba, T. Okura, K. Hattori, Y. Nukada, K. Akita, F. Tanabe, K. Konishi, M. Micallef, M. Fujii, et al. 1996. Cloning of the cDNA for human IFN-γ-inducing factor, expression in *Escherichia coli*, and studies on the biologic activities of the protein. *J. Immunol. 156:4274.*
16. Walter, P., and A. E. Johnson. 1994. Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. *Annu. Rev. Cell Biol. 10:87.*
17. Singer, I. I., S. Scott, G. L. Hall, G. Limjuco, J. Chin, and J. A. Schmidt. 1988. Interleukin 1-β is localized in the cytoplasmic ground substance but is largely absent from the Golgi apparatus and plasma membrane of stimulated monocytes. *J. Exp. Med. 167:389.*
18. Hogquist, K. A., M. A. Nett, E. R. Unanue, and D. D. Chaplin. 1991. Interleukin-1 is processed and released during apoptosis. *Proc. Natl. Acad. Sci. USA 88:8485.*
19. Hogquist, K. A., E. R. Unanue, and D. D. Chaplin. 1991. Release of IL-1 from mononuclear phagocytes. *J. Immunol. 147:2181.*
20. Perregaux, D., J. Barberia, A. J. Lanzetti, K. F. Geoghegan, T. J. Carty, and C. A. Gabel. 1992. IL-1β maturation: evidence that mature cytokine formation can be induced specifically by nigericin. *J. Immunol. 149:1294.*
21. Perregaux, D. G., R. E. Laliberte, and C. A. Gabel. 1996. Human monocyte interleukin-1β posttranslational processing. *J. Biol. Chem. 271:29830.*
22. Bhakdi, S., M. Huhly, S. Korom, and G. Schmidt. 1990. Effects of *Escherichia coli* hemolysin on human monocytes. *J. Clin. Invest. 85:1746.*
23. Perregaux, D. G., L. Svensson, and C. A. Gabel. 1996. Tenidap and other anion transport inhibitors disrupt cytolytic T lymphocyte-mediated IL-1β post-translational processing. *J. Immunol. 157:57.*
24. Laliberte, R., D. G. Perregaux, L. Svensson, C. J. Pazoles, and C. A. Gabel. 1994. Tenidap modulates cytoplasmic pH and inhibits anion transport in vitro. II. Inhibition of IL-1β production from ATP-treated monocytes and macrophages. *J. Immunol. 153:2168.*
25. Ferrari, D., P. Chiozzi, S. Falzoni, M. Dal Susino, L. Melchiorri, O. R. Baricordi, and F. Di Virgilio. 1997. Extracellular ATP triggers IL-1β release by activating the purinergic P2Z receptor of human macrophages. *J. Immunol. 159:1451.*
26. Grahames, C. B. A., A. D. Michel, I. P. Chessell, and P. P. A. Humphrey. 1999. Pharmacological characterization of ATP- and LPS-induced IL-1β release in human monocytes. *Br. J. Pharmacol. 127:1915.*
27. Surprenant, A., F. Rassendren, E. Kawashima, R. A. North, and G. Buell. 1996. The cytolytic P_{2Z} receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). *Science 272:735.*
28. Rassendren, F., G. N. Buell, C. Virginio, G. Collo, R. A. North, and A. Surprenant. 1997. The permeabilizing ATP receptor, $P2X_7$. *J. Biol. Chem. 272:5482.*
29. Michel, A. D., I. P. Chessell, A. D. Hibell, J. Simon, and P. P. A. Humphrey. 1998. Identification and characterization of an endogenous $P2X_7$ (P2Z) receptor in CHO-K1 cells. *Br. J. Pharmacol. 125:1194.*
30. Buisman, H. P., T. H. Steinberg, J. Fischbarg, S. C. Silverstein, S. A. Vogelzang, C. Ince, D. L. Ypey, and P. C. J. Leijh. 1988. Extracellular ATP induces a large nonselective conductance in macrophage plasma membranes. *Proc. Natl. Acad. Sci. USA 85:7988.*
31. Steinberg, T. H., A. S. Newman, J. A. Swanson, and S. C. Silverstein. 1987. ATP^{4-} permeabilizes the plasma membrane of mouse macrophages to fluorescent dyes. *J. Biol. Chem. 262:8884.*
32. Wiley, J. S., C. E. Gargett, W. Zhang, M. B. Snook, and G. P. Jamieson. 1998. Partial agonists and antagonists reveal a second permeability state of human lymphocyte P2Z/$P2X_7$ channel. *Am. J. Physiol. 275:C1224.*
33. Di Virgilio, F. 1995. The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death. *Immunol. Today 16:524.*
34. Perregaux, D. G., and C. A. Gabel. 1998. Post-translational processing of murine IL-1: evidence that ATP-induced release of IL-1α and IL-1β occurs via a similar mechanism. *J. Immunol. 160:2469.*
35. Collo, G., S. Neidhart, E. Kawashima, M. Kosco-Vilbois, R. A. North, and G. Buell. 1997. Tissue distribution of the $P2X_7$ receptor. *Neuropharmacology 36:1277.*
36. Ferrari, D., P. Chiozzi, S. Falzoni, S. Hanau, and F. Di Virgilio. 1997. Purinergic modulation of interleukin-1β release from microglial cells stimulated with bacterial endotoxin. *J. Exp. Med. 185:579.*
37. Griffiths, R. J., E. J. Stam, J. T. Downs, and I. G. Otterness. 1995. ATP induces the release of IL-1 from LPS-primed cells in vivo. *J. Immunol. 154:2821.*
38. Walev, I., K. Reske, M. Palmer, A. Valeva, and S. Bhakdi. 1995. Potassium-inhibited processing of IL-1β in human monocytes. *EMBO J. 14:1607.*
39. Laliberte, R. E., D. G. Perregaux, P. McNiff, C. A. Gabel. 1997. Human monocyte ATP-induced IL-1β posttranslational processing is a dynamic process dependent on in vitro growth conditions. *J. Leukocyte Biol. 62:227.*
40. de Bont, E. S. J. M., A. E. Niemarkt, R. Y. J. Tamminga, J. L. L. Kimpen, W. A. Kamps, and L. H. M. F. de Leij. 1996. Lipopolysaccharide-induced cytokine production in peripheral blood mononuclear cells: intracellular localization of tumor necrosis factor α and interleukin 1β detected with three-color immunofluorescence technique. *Histochem. Cell Biol. 106:593.*
41. Goto, M., M. Fujisawa, A. Yamada, T. Okabe, F. Takaku, M. Sasano, and K. Nishioka. 1990. Spontaneous release of angiotensin converting enzyme and interleukin 1β from peripheral blood monocytes from patients with rheumatoid arthritis under serum-free condition. *Ann. Rheum. Dis. 49:172.*
42. Barkely, D. E. H., M. Feldmann, and R. N. Maini. 1990. Cells with dendritic morphology and bright interleukin-1α staining circulate in the blood of patients with rheumatoid arthritis. *Clin. Exp. Immunol. 80:25.*
43. Baumgartner, R. A., K. Yamada, V. A. Deramo, and M. A. Beaven. 1994. Secretion of TNF from a rat mast cell line is a brefeldin A-sensitive and a calcium/protein kinase C-regulated process. *J. Immunol. 153:2609.*
44. Cornbleet, P. J., D. Myrick, S. Judkins, and R. Levy. 1992. Evaluation of the Cell-Dyn 3000 differential. *Am. J. Clin. Pathol. 98:603.*
45. Ranjeny, T., L. S. Davis, and P. E. Lipsky. 1993. Comparative accessory cell function of human peripheral blood dendritic cells and monocytes. *J. Immunol. 151:6840.*
46. Van Der Poll, T., S. M. Coyle, L. L. Moldawer, and S. F. Lowry. 1996. Changes in endotoxin-induced cytokine production by whole blood after in vivo exposure of normal humans to endotoxin. *J. Infect. Dis. 174:1356.*
47. Fenton, M. J., B. D. Clark, K. L. Collins, A. C. Webb, and P. E. Auron. 1987. Transcriptional regulation of the human prointerleukin 1β gene. *J. Immunol. 138:3972.*
48. Dinarello, C. A. 1998. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int. Rev. Immunol. 16:457.*
49. Newton, R. C. 1986. Human monocyte production of interleukin-1: parameters of the induction of interleukin-1 secretion by lipopolysaccharides. *J. Leukocyte Biol. 39:299.*
50. Puren, A. J., G. Fantuzzi, and C. A. Dinarello. 1999. Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1β are differentially regulated in human blood mononuclear cells and mouse spleen cells. *Proc. Natl. Acad. Sci. USA 96:2256.*
51. Herzyk, D. J., A. E. Berger, J. N. Allen, and M. D. Wewers. 1992. Sandwich ELISA formats designed to detect 17-kDa IL-1β significantly underestimate 35-kDa IL-1β. *J. Immunol. Meth. 148:243.*
52. Hickman, S. E., J. El Khoury, S. Greenberg, I. Schieren, and S. C. Silverstein. 1994. P2Z adenosine triphosphate receptor activity in cultured human monocyte-derived macrophages. *Blood 84:2452.*
53. Falzoni, S., M. Munerati, D. Ferrari, S. Spisani, S. Moretti, and F. Di Virgilio. 1995. The purinergic P2Z receptor of human macrophage cells. *J. Clin. Invest. 95:1207.*
54. Humphreys, B. D., and G. R. Dubyak. 1996. Induction of the P2Z/$P2X_7$ nucleotide receptor and associated phospholipase D activity by lipopolysaccharide and IFN-γ in the human THP-1 monocytic cell line. *J. Immunol. 157:5627.*
55. Humphreys, B. D., C. Virginio, A. Surprenant, J. Rice, and G. R. Dubyak. 1998. Isoquinolines as antagonists of the $P2X_7$ nucleotide receptor: high selectivity for the human versus rat receptor homologues. *Mol. Pharmacol. 54:22.*
56. Schilling, W. P., T. Wasylyna, G. R. Dubyak, B. D. Humphreys, and W. G. Sinkins. 1999. Maitotoxin and P2Z/$P2X_7$ purinergic receptor stimulation activate a common cytolytic pore. *Am J. Physiol. 277:C766.*
57. Chused, T. M., S. Apasov, S., and M. Sitkovsky. 1996. Murine T-lymphocytes modulate activity of an ATP-activated P2Z-type purinoceptor during differentiation. *J. Immunol. 157:1371.*
58. Wiley, J. S., and G. R. Dubyak. 1989. Extracellular adenosine triphosphate increases cation permeability of chronic lymphocytic leukemic lymphocytes. *Blood 1989:1316.*
59. Baricordi, O. R., D. Ferrari, L. Melchiorri, P. Chiozzi, S. Hanau, E. Chiari, M. Rubini, and F. Di Virgilio. 1996. An ATP-activated channel is involved in mitogenic stimulation of human T-lymphocytes. *Blood 87:682.*
60. Wiley, J. S., C. E. Gargett, Z. Zhang, M. B. Snook, and G. P. Jamieson. 1998. Partial agonists and antagonists reveal a second permeability state of human lymphocyte P2Z/$P2X_7$ channel. *Am. J. Physiol. 275:C1224.*
61. Wiley, J. S., R. Chen, and G. P. Jamieson. 1993. The ATP^{4-} receptor-operated channel (P2Z class) of human lymphocytes allows Ba^{2+} and ethidium^{+} uptake: inhibition of fluxes by suramin. *Arch. Biochem. Biophys. 305:54.*
62. Lynch, E. A., C. A. Dinarello, and J. G. Cannon. 1994. Gender differences in IL-1α, IL-1β, and IL-1 receptor antagonist secretion from mononuclear cells and uninary excretion. *J. Immunol. 153:300.*
63. Chin, J., and M. J. Kostura. 1993. Dissociation of IL-1β synthesis and secretion in human blood monocytes stimulated with bacterial cell wall products. *J. Immunol. 151:5574.*