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BRIEF REVIEWS

The $P2X_7$ Receptor: A Key Player in IL-1 Processing and Release[1]

Davide Ferrari,[2][*,†] Cinzia Pizzirani,[*,†] Elena Adinolfi,[*,†] Roberto M. Lemoli,[‡] Antonio Curti,[‡] Marco Idzko,[§] Elisabeth Panther,[§] and Francesco Di Virgilio*[*,†]

Human IL-1 family proteins are key mediators of the host response to infections, injury, and immunologic challenges. The mechanism by which IL-1 activates proinflammatory responses in target cells, and the plasma membrane receptors involved, is fairly well known. This has led to the development of innovative drugs that block IL-1 downstream to its synthesis and secretion. On the contrary, the mechanism of IL-1 and other IL-1 family members (e.g., IL-18) maturation and release is incompletely understood. Accruing evidence points to a plasma membrane receptor for extracellular ATP, the $P2X_7$ receptor, as a key player in both processes. A deeper understanding of the mechanism by which the $P2X_7$ receptor triggers IL-1 maturation and exteriorization may suggest novel avenues for the treatment of inflammatory diseases and provide a deeper insight in the fundamental mechanism of protease activation and cellular export of proteins lacking a leader sequence. The Journal of Immunology, 2006, 176: 3877–3883.

Interleukin-1β is a key mediator of host response to infections and a primary cause of inflammation (1, 2). Bacterial products (e.g., LPS) and host-derived inflammatory factors cause the synthesis of IL-1β in the form of a biologically inactive procytokine (molecular mass, 31 kDa) that remains dispersed in the cytosol until a second stimulus drives processing and release of the 17-kDa active form. IL-1α and IL-18, two additional members of the IL-1 superfamily with proinflammatory activity, are also synthesized as leaderless procytokines and accumulate in the cell cytoplasm in the absence of a maturation-promoting second stimulus (1, 3). Another member of the IL-1 superfamily, IL-1 receptor antagonist (IL-1Ra),[3] is also synthesized and released in parallel to IL-1β, IL-1α, and IL-18 (4). IL-1Ra binds to type I IL-1 receptor (IL-1RI) and blocks IL-1-dependent signal transduction, thus functioning as an endogenous, IL-1-selective inhibitor of inflammation (5).

In vivo, IL-1β is elevated during infections and in several chronic inflammatory diseases such as arthritis, scleroderma, systemic lupus erythematosus, vasculitis, sepsis, septic shock, and in the presence of atherosclerotic lesions leading to myocardial infarction (2). However, the in vitro process of IL-1β release is very inefficient, as activated monocytes release into the extracellular space $< 10\%$ of the IL-1β accumulated intracellularly (6). This finding has led to postulate that IL-1β release requires two stimuli (two hits), the first one driving gene transcription and pro-IL-1β accumulation and the second one processing and release.

In recent years, increasing attention has been paid to extracellular ATP as a candidate danger signal locally released at the inception of inflammation (7, 8). One of the most striking features of this nucleotide is its unmatched ability to promote massive release of mature IL-β from LPS-primed mononuclear phagocytes and other cell types (9). ATP-driven maturation and release of IL-1β are specifically mediated by the $P2X_7$ receptor ($P2X_7R$) for extracellular ATP (10–12). The molecular dissection of this novel pathway for cytokine release may disclose new targets for anti-inflammatory therapy.

IL-1

IL-1, the product of a member of a superfamily of related genes, some of which encode proteins not involved in inflammation (2), causes the accumulation of arachidonic acid metabolites, up-regulates inducible NO synthase, and sustains NO production. Furthermore, it enhances expression of adhesion molecules on endothelial cells, promotes extravasation of leukocytes, modulates muscle metabolism, and induces fever (2, 13). IL-1 is also a key activator of osteoclasts, thus causing bone resorption (14).

IL-1α and IL-1β share low sequence homology (20–30%) but high three-dimensional similarity. Upon cell activation,

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[3] Abbreviations used in this paper: IL-1Ra, IL-1 receptor antagonist; IL-1RI, type I IL-1 receptor; casp, caspase; procasp, procaspase; ASC, apoptosis-associated speck-like protein containing a CARD; IL-1R-AcP, IL-1R accessory protein; DC, dendritic cell; SNP, single nucleotide polymorphism; $P2X_7R$, $P2X_7$ receptor.

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typically via plasma membrane TLRs, IL-1α and IL-1β are translated as 31-kDa leaderless secretory proteins (pro-IL-1α and pro-IL-1β). Although IL-1α is already active in this form, IL-1β needs a proteolytical step to generate the 17-kDa mature cytokine. Pro-IL-1β can be processed in the extracellular milieu by several proteases activated during inflammation, whereas the IL-1β-converting enzyme, later named caspase (casp)-1, is the key protease responsible for intracellular IL-1β processing (15, 16). Recently, IL-33, an IL-1β-like cytokine acting at the ST2 receptor, has also been shown to be processed by casp-1 (17). In resting cells, casp-1 is normally present as an inactive 45-kDa precursor, procaspase (procasp)-1. Proteolytical activation of procasp-1 occurs in a multimeric specialized structure named "IL-1β inflammasome protein complex" (18) comprising at least four different proteins (casp-1, casp-5, apoptosis-associated speck-like protein containing a CARD (ASC), and NACHT-, LRR-, and PYD-containing protein) that transiently oligomerize upon cell activation (19). The mechanism of inflammasome assembly and activation is a focus of intense investigation.

IL-1α and IL-1β bind to the same receptor named IL-1RI. Upon IL-1 binding, IL-1RI recruits the accessory protein IL-1R-AcP, a member of the Ig superfamily, and initiates a stimulatory signal transduction cascade. A decoy receptor is also present, IL-1RII, that is unable to complex with IL-1R-AcP and to generate a biological signal (20). IL-1RII competes with IL-1RI to down-modulate IL-1 activity (21).

IL-1Ra

The IL-1Ra is secreted in response to the same stimuli that drive IL-1 release and binds to IL-1RI and IL-1RII (4). However, IL-1Ra lacks the IL-1R-AcP interacting domain, thus its binding results in inhibition of the IL-1 signaling cascade (22). IL-1Ra is generally secreted without requiring maturation, its function being to silence IL-1-dependent cell activation. Different IL-1Ra isoforms have been identified, of which three are intracellular (23).

The IL-1Ra knockout mouse spontaneously develops diseases similar to human rheumatoid arthritis and arteritis (24, 25). Administration of anakinra, a recombinant form of IL-1Ra, reduces local inflammation of joints and bone erosions in patients with rheumatoid arthritis (26, 27).

IL-18

IL-18 shares structural features with IL-1 (3). Like IL-1, IL-18 also lacks a signal peptide, and it is synthesized as a biologically 24-kDa inactive precursor (pro-IL-18), which is converted by casp-1 to its mature 18-kDa form (28, 29). In contrast to IL-1β, IL-18 is not an endogenous pyrogen (2). IL-18 binds to an $\alpha\beta$ heterodimeric receptor inducing the synthesis of other proinflammatory cytokines (IL-6, IL-8, TNF-α, IL-1β, and IFN-γ), CD95 ligand, and several chemokines. It also increases the expression of ICAM-1 and VCAM-1 on endothelial cells (2). IL-18 is secreted by a variety of cells, including epithelial cells, keratinocytes, synovial fibroblasts, monocytes, macrophages, and dendritic cells (DCs) (30). Like IL-1, IL-18 behaves as an angiogenic factor through increased expression of vascular endothelial growth factor.

The P2X$_7$R for extracellular ATP

Basically all cell types express plasma membrane receptors for extracellular nucleotides named P2 receptors. As of now, 15 members have been cloned and classified into two subfamilies: P2Y and P2X. P2YRs are G protein coupled, whereas P2XRs are cation-selective channels (31, 32). The P2X$_7$R (Fig. 1) is a most peculiar receptor/channel in that sustained activation by extracellular ATP causes the formation of a reversible plasma membrane pore permeable to hydrophilic solutes of molecular mass up to 900 Da (33, 34). Pore formation is essential for P2X$_7$R-stimulated IL-1 release (35).

The *P2X$_7$R* gene is located at position 12q24 and encodes a 595-aa long polypeptide with two transmembrane stretches, a bulky extracellular domain and N- and C-terminal residues both on the cytoplasmic side of the plasma membrane (34, 36). The extracellular loop contains 10 cysteine residues that may form disulfide bonds and a sequence stretch (aa 170–330) likely containing six-stranded β-pleated sheets (37). This region has been proposed to host the ATP binding site (38). The

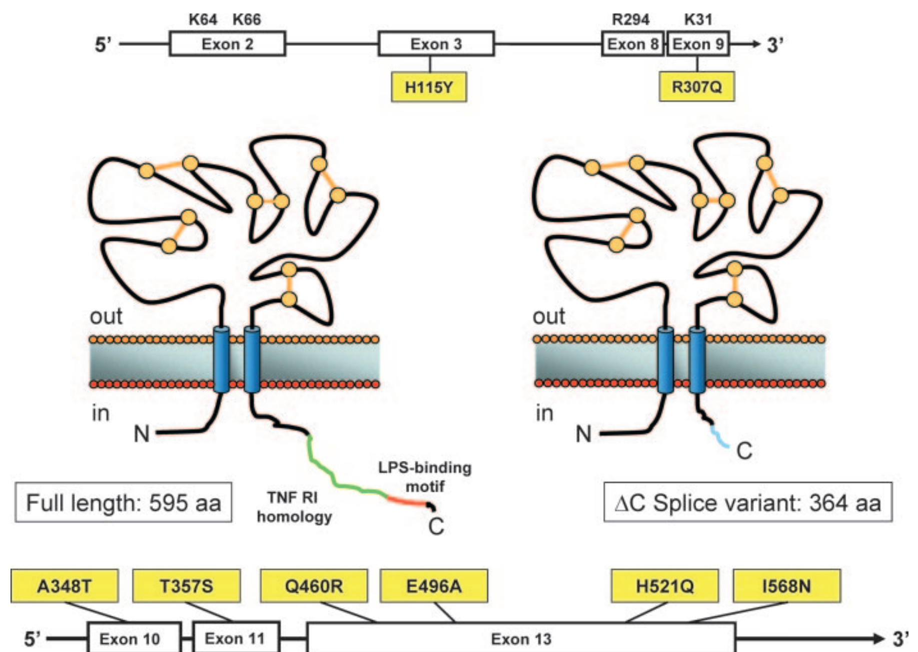

FIGURE 1. The P2X$_7$R: membrane topology and aa substitutions caused by the main SNPs so far described. The full-length and truncated (ΔC) splice variant of the P2X$_7$R are shown. The truncated form lacks almost the entire COOH tail (249 aa, green, red, and short black traits) but bears an extra 18 aa (light blue trait) due to inclusion of the intron between exons 10 and 11 (94). TNFRI homology domain (green) and putative LPS-binding region (red) are shown. Cysteine residues forming putative disulfide bridges are also shown. Amino acid changes are highlighted in yellow boxes, whereas residues involved in ATP binding in exons 2, 3, 8, and 9 are indicated in black.

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main structural distinctive feature of the $P2X_7R$ is a long C-terminal tail (244 aa) harboring multiple potential protein and lipid interaction motifs (see Fig. 1) (39). Although other receptors of the P2X subfamily have been shown to form functional hetero-oligomers, the $P2X_7$ subunits seem to associate only with each other to form homomeric $P2X_7R$ (40).

Unique in the P2XR subfamily, $P2X_7R$ is highly polymorphic. More than 260 single nucleotide polymorphisms (SNPs) have been described in the human *P2X₇R* gene, but only a few have been functionally characterized. Wiley and coworkers (41–43) have identified four loss-of-function single amino acid substitutions, three located in the cytoplasmic tail of the receptor (T357S, E496A, and I568N), and one in the putative ATP binding site (R307Q) (38). Our group has characterized the first gain-of-function polymorphism so far identified (H155Y) (44). Wiley and coworkers (45) have also described a splice site mutation in the first intron of the $P2X_7R$ gene that produces a null allele. Polymorphisms in the promoter region of the gene have also been identified (46). No convincing disease association has been so far demonstrated for these SNPs.

The mechanism of pore formation by the $P2X_7R$ is a matter of debate: while some investigators hold the conservative opinion that pore formation is due to an ATP-dependent increase in size of the $P2X_7R$ channel itself (47), others believe that the pore is a separate molecular structure activated by the $P2X_7R$ (48, 49). Early studies by Dubyak and coworkers (48, 49) suggested that it might coincide with the plasma membrane pore activated by the sea toxin maitotoxin. More recently, Elliott et al. (50) have hypothesized that $P2X_7R$-dependent transitions in plasma membrane permeability might involve the modulation of the multidrug transporter P-glycoprotein. Whichever the molecular basis of pore formation, there is no doubt that $P2X_7R$ activation causes a massive upset of cytoplasmic ion homeostasis (51). Because the $P2X_7R$ is nondesensitizing, the pore stays open as long it is bound by ATP. Removal of the nucleotide, by rinsing or apyrase-catalyzed hydrolysis, causes pore closure, thus allowing a reversible plasma membrane permeabilization. Should ATP stimulation be prolonged (over 15–30 min in most cell types), the cells become irreversibly injured and committed to death (52).

The $P2X_7R$ and IL-1 release

LPS, the best known inducer of IL-1 secretion, is an incomplete stimulus because it causes massive accumulation of pro-IL-1β in the cytosol while acting as only a very weak stimulator of IL-1β maturation and externalization (6). This has led investigators to postulate a second stimulus to trigger efficient IL-1β conversion and release. Early observations showed that extracellular ATP was a strong IL-1β-releasing agent (53–55). Although at that time the identity of the plasma membrane receptor(s) involved and the signal transduction cascade activated was unknown, the massive efflux of intracellular K^+ triggered by extracellular ATP suggested a mechanism based on cytoplasmic K^+ depletion (51, 54, 56). Experiments performed in vitro and in vivo in $P2X_7R^{-/-}$ mice conclusively identified the receptor responsible for ATP-dependent IL-1β release as the $P2X_7R$ (10–12, 57, 58).

The ATP effect is very fast, as externalization of mature IL-1β is near maximal within 10–20 min. The main IL-1β released form is the 17 kDa, but sustained ATP stimulation can also cause release of the 31-kDa form and of cytoplasmic markers suggestive of a late nonspecific damage of the plasma membrane (11, 54). However, if care is taken to use ATP concentrations in the 0.5–1 mM range and short incubation times (15 min), a selective release of mature IL-1β occurs and cells fully recover from the ATP challenge (11). Furthermore, it has been shown that cell lysis per se is unable to support pro-IL-1β conversion (59).

It is currently debated whether extracellular ATP needs LPS priming to activate casp-1. Although until recently it was thought that priming was unnecessary (60, 61), two novel studies suggest that LPS pretreatment might indeed be needed to achieve substantial generation of casp-1 activation fragments in mouse macrophages (62, 63). Furthermore, blockade by YVAD-CHO shows that casp-1 activity is necessary for $P2X_7R$-dependent release of mature IL-1β (Refs. 60 and 61 but see Ref. 64). The role of K^+ efflux in supporting $P2X_7R$-dependent IL-1β processing and release seems to be crucial as both processes are suppressed by inhibition of intracellular K^+ efflux (11, 54, 61, 65, 66). Conversely, release of IL-1β is enhanced in sucrose-containing medium, a condition in which K^+ efflux is strongly potentiated (11, 61). Incidentally and in further support of the view that $P2X_7R$-dependent IL-1β secretion does not require cell lysis, in sucrose medium, ATP-stimulated release of cytoplasmic markers is negligible (11, 67). Mutations in the human $P2X_7R$ that prevent pore formation and reduce ATP-stimulated K^+ efflux (e.g., the E496A substitution) inhibit IL-1β release (35). Interestingly, this inhibitory effect is more marked in isolated macrophages than in whole blood (68), suggesting that in the presence of blood cells additional stimulatory factors are released. Altogether, there is consensus that the drastic upset of K^+ homeostasis caused by $P2X_7R$ opening has a key role in triggering IL-1β maturation, whereas other intracellular ions (e.g., Ca^{2+}) seem to be more involved in IL-1β release (66, 69). This view is also supported by the casp-1 activating effect of nigericin-induced K^+ depletion (70). A recent article (62) by Dixit's laboratory shows that mice deficient in the adaptor protein ASC fail to process pro-IL-1β in response to ATP, thus suggesting that $P2X_7R$-dependent casp-1 cleavage may require assembly of a fully functional inflammasome. How the change in intracellular K^+ concentration drives this process is an open question. On the other hand, a direct physical interaction of the $P2X_7R$ with the inflammasome is as yet undocumented.

IL-1β is a leaderless protein that does not appear to be contained within classical exocytotic vesicles. Thus, how it is externalized has long been a mystery. In the last few years experiments by Rubartelli and coworkers (66) and Surprenant and colleagues (71) have shed light on this issue suggesting a vesicular mechanism (Fig. 2). However, there are two crucial differences in the pathways proposed by the two groups: Rubartelli suggests that IL-1β is accumulated into endocytic vesicles (secretory lysosomes) together with casp-1, then a $P2X_7R$-induced loss of intracellular K^+ activates phosphatidylcholine-specific phospholipase C, which in turn causes an increase in cytosolic Ca^{2+}, Ca^{2+}-dependent phospholipase A_2 activation, and exocytosis of the IL-1β-containing lysosomes. According to this model, casp-1 activation and IL-1β processing are triggered by the K^+ loss-stimulated activation of a Ca^{2+}-independent phospholipase A_2 within the lysosomes.

In the model proposed by Surprenant, upon $P2X_7R$-mediated macrophage activation, IL-1β is packaged into small

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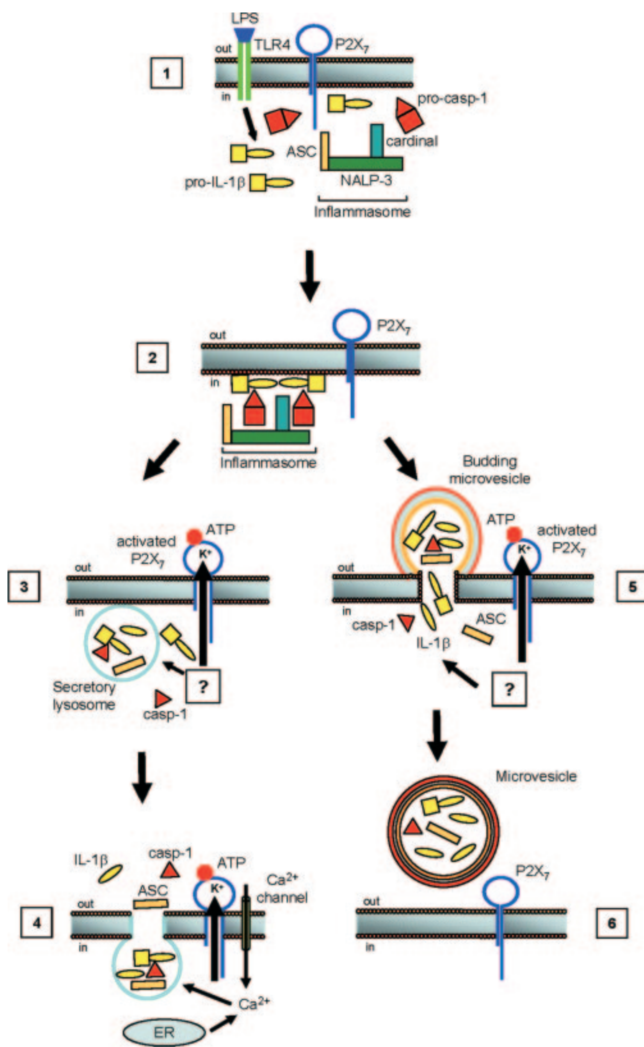

FIGURE 2. Pathways for P2X$_7$-stimulated IL-1β cleavage and release. Activation of TLRs by LPS causes cytoplasmic accumulation of pro-IL-1β and aggregation of the inflammasome components (step 1). Pro-IL-1β and the inflammasome localize below the inner leaflet of the plasma membrane (step 2). Activation of the P2X$_7$R triggers K^+ efflux, which in turn drives loading of some components of the inflammasome (e.g., pro-IL-1β, casp-1, and ASC) into secretory lysosomes (66). During this process, procasp-1 is cleaved, and pro-IL-1β is converted into mature IL-1β (step 3). The secretory lysosome content is then secreted via a process requiring P2X$_7$R-dependent K^+ efflux, Ca^{2+} increase, activation of phosphatidylcholine-specific phospholipase C, and both Ca^{2+}-independent phospholipase A_2 and Ca^{2+}-dependent phospholipase A_2 (step 4). An alternative model hypothesizes that after the inflammasome has aggregated below the inner leaflet of the plasma membrane (step 2), activation of the P2X$_7$R triggers budding of small membrane blebs (microvesicles, notice phosphatidylserine flip in the outer leaflet, red) that trap some of the inflammasome components (e.g., pro-IL-1β, casp-1, and ASC). During this process, which is driven by the K^+ loss, procasp-1 is cleaved, and pro-IL-1β is converted into mature IL-1β (step 5). Eventually, plasma membrane blebs pinch off and diffuse into the pericellular space (step 6).

plasma membrane blebs that are released into the extracellular space as microvesicles of size ranging from 200 nm to 1 mm. These microvesicles are akin to the tissue factor-containing microparticles released from various cell types (72) and different from the large plasma membrane blebs that are also produced in cells stimulated via the P2X$_7$R (71, 73). Microvesicle budding and release are preceded by phosphatidylserine flip and loss of membrane asymmetry (71), a change in the membrane phospholipid structure that might have a relevant signaling function (50). Recent experiments have shown that a vesicle-mediated mechanism for IL-1β release is also present in mouse microglia and human DCs (Ref. 74; C. Pizzirani and F. Di Virgilio, submitted for publication). In DCs, casp-1 and -3 are also loaded into the vesicles, thus providing a mechanism for intravesicular pro-IL-1β processing.

The microvesicle model proposed by Surprenant raises an obvious criticism: how does mature IL-1β get through the microvesicle membrane and reaches the extracellular space to activate IL-1Rs? Recent observations from Verderio's laboratory and from our own group provide a possible answer: microvesicles shed from microglia and DCs, express the P2X$_7$R (together with several other markers), and lyse when exposed to extracellular ATP (Ref. 74; C. Pizzirani and F. Di Virgilio, submitted for publication). Thus, it can be hypothesized that once the microvesicles approach the plasma membrane of the target cells, where the ATP concentration is higher than in the bulk solution due to active cellular release (75), the P2X$_7$R is activated, the microvesicles lyse, and IL-1β is released. In both the Rubartelli and Surprenant models, P2X$_7$R activation is crucial, but how and if this receptor is activated under physiological conditions is as yet an open question.

P2X$_7$R activation by extracellular ligands

The only known physiological activator of the P2X$_7$R is ATP. Scattered evidence suggests that ADP, but not other nucleotides, may also cause IL-1β release via P2X$_7$R, but it is likely that this needs priming with ATP (76, 77). Since the cytoplasmic ATP concentration is in the millimolar range, acute cell injury or death will cause massive ATP release into the extracellular milieu. However, there is compelling evidence for nonlytic ATP secretion via as yet unidentified pathways activated by host- or pathogen-derived factors (76, 78–81). Measurements performed with membrane-bound probes in platelets, human neuroblastoma cells, and P2X$_7$R-transfected HEK293 cells show that the ATP concentration at the plasma membrane surface is much higher than in the bulk solution and may range from 20 to 200 μm (75, 82). This suggests that ATP levels sufficient to activate the P2X$_7$R may be reached in the pericellular space. In addition, proinflammatory cytokines or bacterial products up-regulate P2X$_7$R expression and increase its sensitivity to extracellular ATP (83–85). In principle, pericellular ATP levels can also be increased by ecto-ATPase inhibition. It has been recently shown that Abs raised against a surface expressed ATPase known as regeneration and tolerance factor potentiate ATP-stimulated IL-1β release from macrophages (86). In mouse, but not in human, thymocytes ADP-ribosylation due to extracellular NAD can also activate the P2X$_7$R, although it is not clear whether this mechanism is operative in macrophages (87). More recently, an antimicrobial peptide derived from human cathelicidin (LL37) was reported to directly activate the P2X$_7$R and trigger IL-1β secretion (80). Thus, it is likely that as our knowledge of the P2X$_7$R expands, we will find additional physiologically relevant ligands for this receptor.

P2X$_7$R-dependent secretion of other IL-1 family members

IL-1α, as with IL-1β, is retained mostly within the cytoplasm unless extracellular ATP is added (88). Stimulation of IL-1α release occurs via the P2X$_7$R and follows a time course similar to that of IL-1β. Both pro-IL-1α and mature IL-1α are released.

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The physiological meaning of $P2X_7$R-dependent pro-IL-1α cleavage is not clear because this cytokine, in contrast to pro-IL-1β, is a full agonist at IL-1Rs.

In response to ATP, macrophages and endothelial cells (HUVECs) also release IL-1Ra. In macrophages, but not in HUVECs, release occurs via $P2X_7$R-stimulated microvesicle release (89). In monocytes, $P2X_7$R-stimulated release of IL-18 occurs with a time course similar to that of IL-1β, requires priming with LPS, and is prevented by the $P2X_7$R E496A loss-of-function polymorphism (64, 90).

IL-1 and ATP as danger signals

There is increasing awareness that stressed cells release soluble signals (danger signals) that activate the immune system (7). Such signals can be classified into constitutive and inducible. While IL-1 is an obvious inducible danger signal, intracellular nucleotides, and among them chiefly ATP, are good candidates to the role of ubiquitous constitutive danger signals (Fig. 3) (7, 8, 91). Several features make ATP well suited to the role of danger signal: high intracellular:extracellular concentration ratio, hydrophilicity, ubiquitous expression of very efficient degrading systems (ecto-ATPases), presence of specific receptors with widely different affinity, and strong DCs maturating activity (8, 92).

Inflammation is a complex response generated by an interacting network of stimulatory and inhibitory signals. Immune cells primed by soluble factors produced by infections or tissue damage may or may not progress to a full-activated phenotype depending on the additional signals that they receive by neighboring cells (93). We think that the $P2X_7$R may act as a "sensor of danger" that monitors the release of danger signals (ATP) at inflammation sites and drives mononuclear phagocytes primed by bacterial products into fully activated inflammatory effectors (IL-1-secreting cells). Thus, in the coordinate response to pathogens, $P2X_7$R might be a "checkpoint" where the choice is made to progress to a full-blown response or put inflammation on hold.

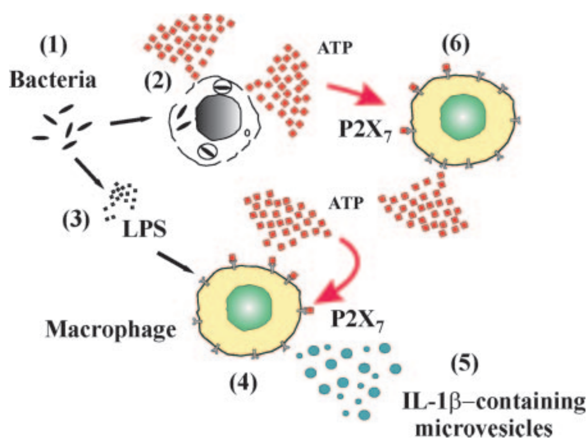

FIGURE 3. Extracellular ATP as a diffusible danger signal generated during bacterial infections. Bacteria enter tissues (step 1) and may directly injure cells and cause ATP release (step 2). Bacterial products (e.g., LPS, step 3) may also cause ATP release (step 4). Extracellular ATP diffuses to activate neighboring cells by paracrine or autocrine pathways (step 4). Macrophages stimulated by LPS and ATP release IL-1-containing microvesicles (step 5). The $P2X_7$R may function as an amplification device to spread the ATP wave as its activation triggers further ATP release (step 6) (75).

Perspectives

The more we learn about inflammation and its role in pathogen fighting, the more we appreciate that living organisms need to exert a tight control on this process to harvest its most beneficial effects. IL-1 family members have a central role in inflammatory diseases. Several novel molecular inhibitors targeting sites downhill to IL-1 synthesis have been introduced in the clinic or are in preclinical trials (e.g., anti-IL-1β or IL-1RI Abs, rIL-1Ra or IL-1Trap). Now, the many points of control of IL-1 synthesis, processing, and release suggest alternative strategies. Cell permeant casp-1 inhibitors are a viable option, but in the light of the recent discoveries on the role of the $P2X_7$R, we anticipate that also blockers of $P2X_7$R may have a future as anti-inflammatory drugs.

References

1. Dinarello, C. A. 1996. Biologic basis for interleukin-1 in disease. *Blood* 87: 2095–2147.
2. Dinarello, C. A. 2002. The IL-1 family and inflammatory diseases. *Clin. Exp. Rheumatol.* 20: S1–S13.
3. Reddy, P. 2004. Interleukin-18: recent advances. *Curr. Opin. Hematol.* 11: 405–410.
4. Arend, W. P., and C. Gabay. 2000. Physiologic role of interleukin-1 receptor antagonist. *Arthritis Res.* 2: 245–248.
5. Dinarello, C. A. 2000. The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. *N. Engl. J. Med.* 343: 732–734.
6. Dinarello, C. A. 2004. Unraveling the NALP-3/IL-1β inflammasome: a big lesson from a small mutation. *Immunity* 20: 243–244.
7. Gallucci, S., and P. Matzinger. 2001. Danger signals: SOS to the immune system. *Curr. Opin. Immunol.* 13: 114–119.
8. Di Virgilio, F. 2005. Purinergic mechanism in the immune system: a signal of danger for dendritic cells. *Purinergic Signal.* 1: 205–209.
9. Di Virgilio, F., P. Chiozzi, D. Ferrari, S. Falzoni, J. M. Sanz, A. Morelli, M. Torboli, G. Bolognesi, and O. R. Baricordi. 2001. Nucleotide receptors: an emerging family of regulatory molecules in blood cells. *Blood* 97: 587–600.
10. Ferrari, D., M. Villalba, P. Chiozzi, S. Falzoni, P. Ricciardi-Castagnoli, and F. Di Virgilio. 1996. Mouse microglial cells express a plasma membrane pore gated by extracellular ATP. *J. Immunol.* 156: 1531–1539.
11. 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: 1451–1458.
12. Solle, M., J. Labasi, D. G. Perregaux, E. Stam, N. Petrushova, B. H. Koller, R. J. Griffiths, and C. A. Gabel. 2001. Altered cytokine production in mice lacking $P2X_7$ receptors. *J. Biol. Chem.* 276: 125–132.
13. Allan, S. M., P. J. Tyrrell, and N. J. Rothwell. 2005. Interleukin-1 and neuronal injury. *Nat. Rev. Immunol.* 5: 629–640.
14. Tanaka, Y., S. Nakayamada, and Y. Okada. 2005. Osteoblasts and osteoclasts in bone remodeling and inflammation. *Curr. Drug Targets. Inflamm. Allergy* 4: 325–328.
15. Hazuda, D. J., J. Strickler, F. Kueppers, P. L. Simon, and P. R. Young. 1990. Processing of precursor interleukin 1β and inflammatory disease. *J. Biol. Chem.* 265: 6318–6322.
16. 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: 768–774.
17. Schmitz, J., A. Owyang, E. Oldham, Y. Song, E. Murphy, T. K. McClanahan, G. Zurawski, M. Moshrefi, J. Qin, X. Li, et al. 2005. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23: 479–490.
18. Martinon, F., K. Burns, and J. Tschopp. 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of pro-IL-β. *Mol. Cell* 10: 417–426.
19. Martinon, F., and J. Tschopp. 2004. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 117: 561–574.
20. Greenfeder, S. A., P. Nunes, L. Kwee, M. Labow, R. A. Chizzonite, and G. Ju. 1995. Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J. Biol. Chem.* 270: 13757–13765.
21. Colotta, F., F. Re, M. Muzio, R. Bertini, N. Polentarutti, M. Sironi, J. G. Giri, S. K. Dower, J. E. Sims, and A. Mantovani. 1993. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* 261: 472–475.
22. Dinarello, C. A. 2000. The role of the interleukin-1 receptor antagonist in blocking inflammation mediated by interleukin-1. *N. Engl. J. Med.* 343: 732–734.
23. Dinarello, C. A. 1994. The interleukin-1 family: 10 years of discovery. *FASEB J.* 8: 1314–1325.
24. Nicklin, M. J. H., D. E. Hughes, J. L. Barton, J. M. Ure, and G. W. Duff. 2000. Arterial inflammation in mice lacking the interleukin 1 receptor antagonist gene. *J. Exp. Med.* 191: 303–312.
25. Horai, R., S. Saijo, H. Tanioka, S. Nakae, K. Sudo, A. Okahara, T. Ikuse, M. Asano, and Y. Iwakura. 2000. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J. Exp. Med.* 191: 313–320.

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26. Bresnihan, B., J. M. varo-Gracia, M. Cobby, M. Doherty, Z. Domljan, P. Emery, G. Nuki, K. Pavelka, R. Rau, B. Rozman, et al. 1998. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *Arthritis Rheum.* 41: 2196–2204.
27. Furst, D. E. 2004. Anakinra: review of recombinant human interleukin-I receptor antagonist in the treatment of rheumatoid arthritis. *Clin. Ther.* 26: 1960–1975.
28. Ghayur, T., S. Banerjee, M. Hugunin, D. Butler, L. Herzog, A. Carter, L. Quintal, L. Sekut, R. Talanian, M. Paskind, et al. 1997. Caspase-1 processes IFN-γ-inducing factor and regulates LPS-induced IFN-γ production. *Nature* 386: 619–623.
29. 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: 206–209.
30. McInnes, I. B., J. A. Gracie, B. P. Leung, X. Q. Wei, and F. Y. Liew. 2000. Interleukin 18: a pleiotropic participant in chronic inflammation. *Immunol. Today* 21: 312–315.
31. Ralevic, V., and G. Burnstock. 1998. Receptors for purines and pyrimidines. *Pharmacol. Rev.* 50: 413–492.
32. Burnstock, G., and G. E. Knight. 2004. Cellular distribution and functions of P2 receptor subtypes in different systems. *Int. Rev. Cytol.* 240: 31–304.
33. Di Virgilio, F. 1995. The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death. *Immunol. Today* 16: 524–528.
34. Surprenant, A., F. Rassendren, E. Kawashima, R. A. North, and G. Buell. 1996. The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X$_7$). *Science* 272: 735–738.
35. Sluyter, R., A. N. Shemon, and J. S. Wiley. 2004. Glu[496] to Ala polymorphism in the P2X$_7$ receptor impairs ATP-induced IL-1β release from human monocytes. *J. Immunol.* 172: 3399–3405.
36. Buell, G. N., F. Talabot, A. Gos, J. Lorenz, E. Lai, M. A. Morris, and S. E. Antonarakis. 1998. Gene structure and chromosomal localization of the human P2X$_7$ receptor. *Receptors Channels* 5: 347–354.
37. Freist, W., J. F. Verhey, W. Stuhmer, and D. H. Gauss. 1998. ATP binding site of P2X channel proteins: structural similarities with class II aminoacyl-tRNA synthetases. *FEBS Lett.* 434: 61–65.
38. Gu, B. J., R. Sluyter, K. K. SkarRatt, A. N. Shemon, L. P. Dao-Ung, S. J. Fuller, J. A. Barden, A. L. Clarke, S. Petrou, and J. S. Wiley. 2004. An Arg[307] to Gln polymorphism within the ATP-binding site causes loss of function of the human P2X$_7$ receptor. *J. Biol. Chem.* 279: 31287–31295.
39. Denlinger, L. C., P. L. Fisette, J. A. Sommer, J. J. Watters, U. Prabhu, G. R. Dubyak, R. A. Proctor, and P. J. Bertics. 2001. Cutting edge: the nucleotide receptor P2X$_7$ contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. *J. Immunol.* 167: 1871–1876.
40. Egan, T. M., J. A. Cox, and M. M. Voigt. 2004. Molecular structure of P2X receptors. *Curr. Top. Med. Chem.* 4: 821–829.
41. Gu, B. J., W. Zhang, R. A. Worthington, R. Sluyter, P. Dao-Ung, S. Petrou, J. A. Barden, and J. S. Wiley. 2001. A Glu[496] to Ala polymorphism leads to loss of function of the human P2X$_7$ receptor. *J. Biol. Chem.* 276: 11135–11142.
42. Wiley, J. S., L. P. Dao-Ung, C. Li, A. N. Shemon, B. J. Gu, M. L. Smart, S. J. Fuller, J. A. Barden, S. Petrou, and R. Sluyter. 2003. An Ile[568] to Asn polymorphism prevents normal trafficking and function of the human P2X$_7$ receptor. *J. Biol. Chem.* 278: 17108–17113.
43. Shemon, A. N., R. Sluyter, S. L. Fernando, A. L. Clarke, L. P. Dao-Ung, K. K. SkarRatt, B. M. Saunders, K. S. Tan, B. J. Gu, S. J. Fuller, et al. 2005. A Thr[357] to Ser polymorphism in homozygous and compound heterozygous subjects causes absent or reduced P2X$_7$ function and impairs ATP-induced mycobacterial killing by macrophages. *J. Biol. Chem.* 281: 2079–2086.
44. Cabrini, G., S. Falzoni, S. L. Forchap, P. Pellegatti, A. Balboni, P. Agostini, A. Cuneo, G. Castoldi, O. R. Baricordi, and F. Di Virgilio. 2005. A His[155] to Tyr polymorphism confers gain-of-function to the human P2X$_7$ receptor of human leukemic lymphocytes. *J. Immunol.* 175: 82–89.
45. SkarRatt, K. K., S. J. Fuller, R. Sluyter, L. P. Dao-Ung, B. J. Gu, and J. S. Wiley. 2005. A 5′ intronic splice site polymorphism leads to a null allele of the *P2X$_7$* gene in 1–2% of the Caucasian population. *FEBS Lett.* 579: 2675–2678.
46. Li, C. M., S. J. Campbell, D. S. Kumararatne, R. Bellamy, C. Ruwende, K. P. McAdam, A. V. Hill, and D. A. Lammas. 2002. Association of a polymorphism in the *P2X$_7$* gene with tuberculosis in a Gambian population. *J. Infect. Dis.* 186: 1458–1462.
47. Di Virgilio, F., O. R. Baricordi, R. Romagnoli, and P. G. Baraldi. 2005. Leukocyte P2 receptors: a novel target for anti-inflammatory and anti-tumor therapy. *Curr. Drug Targets Cardiovasc. Haematol. Disord.* 5: 85–99.
48. 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(4 Pt. 1): C766–C776.
49. Verhoef, P. A., S. B. Kertesy, M. Estacion, W. P. Schilling, and G. R. Dubyak. 2004. Maitotoxin induces biphasic interleukin-1β secretion and membrane blebbing in murine macrophages. *Mol. Pharmacol.* 66: 909–920.
50. Elliott, J. I., A. Surprenant, F. M. Marelli-Berg, J. C. Cooper, R. L. Cassady-Cain, C. Wooding, K. Linton, D. R. Alexander, and C. F. Higgins. 2005. Membrane phosphatidylserine distribution as a non-apoptotic signalling mechanism in lymphocytes. *Nat. Cell Biol.* 7: 808–816.
51. Steinberg, T. H., and S. C. Silverstein. 1987. Extracellular ATP4- promotes cation fluxes in the J774 mouse macrophage cell line. *J. Biol. Chem.* 262: 3118–3122.
52. Di Virgilio, F., P. Chiozzi, S. Falzoni, D. Ferrari, J. M. Sanz, V. Venketaraman, and O. R. Baricordi. 1998. Cytolytic P2X purinoceptors. *Cell Death Differ.* 5: 191–199.
53. Hogquist, K. A., E. R. Unanue, and D. D. Chaplin. 1991. Release of IL-1 from mononuclear phagocytes. *J. Immunol.* 147: 2181–2186.
54. Perregaux, D., and C. A. Gabel. 1994. Interleukin-1β maturation and release in response to ATP and nigericin: evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. *J. Biol. Chem.* 269: 15195–15203.
55. 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: 2821–2828.
56. Di Virgilio, F., D. Ferrari, S. Falzoni, P. Chiozzi, M. Munerati, T. H. Steinberg, and O. R. Baricordi. 1996. P2 purinoceptors in the immune system. *Ciba Found. Symp.* 198: 290–302.
57. Labasi, J. M., N. Petrushova, C. Donovan, S. McCurdy, P. Lira, M. M. Payette, W. Brissette, J. R. Wicks, L. Audoly, and C. A. Gabel. 2002. Absence of the P2X$_7$ receptor alters leukocyte function and attenuates an inflammatory response. *J. Immunol.* 168: 6436–6445.
58. Chessell, I. P., J. P. Hatcher, C. Bountra, A. D. Michel, J. P. Hughes, P. Green, J. Egerton, M. Murfin, J. Richardson, W. L. Peck, et al. 2005. Disruption of the P2X$_7$ purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* 114: 386–396.
59. 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: 1294–1303.
60. Laliberte, R. E., J. Eggler, and C. A. Gabel. 1999. ATP treatment of human monocytes promotes caspase-1 maturation and externalization. *J. Biol. Chem.* 274: 36944–36951.
61. Sanz, J. M., and F. Di Virgilio. 2000. Kinetics and mechanism of ATP-dependent IL-1β release from microglial cells. *J. Immunol.* 164: 4893–4898.
62. Mariathasan, S., K. Newton, D. M. Monack, D. Vucic, D. M. French, W. P. Lee, M. Roose-Girma, S. Erickson, and V. M. Dixit. 2004. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430: 213–218.
63. Kahlenberg, J. M., K. C. Lundberg, S. B. Kertesy, Y. Qu, and G. R. Dubyak. 2005. Potentiation of caspase-1 activation by the P2X$_7$ receptor is dependent on TLR signals and requires NF-κB-driven protein synthesis. *J. Immunol.* 175: 7611–7622.
64. Mehta, V. B., J. Hart, and M. D. Wewers. 2001. ATP-stimulated release of interleukin (IL)-1β and IL-18 requires priming by lipopolysaccharide and is independent of caspase-1 cleavage. *J. Biol. Chem.* 276: 3820–3826.
65. Kahlenberg, J. M., and G. R. Dubyak. 2004. Mechanisms of caspase-1 activation by P2X$_7$ receptor-mediated K^+ release. *Am. J. Physiol.* 286: C1100–C1108.
66. Andrei, C., P. Margiocco, A. Poggi, L. V. Lotti, M. R. Torrisi, and A. Rubartelli. 2004. Phospholipases C and A2 control lysosome-mediated IL-1β secretion: implications for inflammatory processes. *Proc. Natl. Acad. Sci. USA* 101: 9745–9750.
67. Murgia, M., P. Pizzo, T. H. Steinberg, and V. F. Di Virgilio. 1992. Characterization of the cytotoxic effect of extracellular ATP in J774 mouse macrophages. *Biochem. J.* 288: 897–901.
68. Denlinger, L. C., G. Angelini, K. Schell, D. N. Green, A. G. Guadarrama, U. Prabhu, D. B. Coursin, P. J. Bertics, and K. Hogan. 2005. Detection of human P2X7 nucleotide receptor polymorphisms by a novel monocyte pore assay predictive of alterations in lipopolysaccharide-induced cytokine production. *J. Immunol.* 174: 4424–4431.
69. Gudipaty, L., J. Munetz, P. A. Verhoef, and G. R. Dubyak. 2003. Essential role for Ca^{2+} in regulation of IL-1β secretion by P2X$_7$ nucleotide receptor in monocytes, macrophages, and HEK-293 cells. *Am. J. Physiol.* 285: C286–C299.
70. Cheneval, D., P. Ramage, T. Kastelic, T. Szelestenyi, H. Niggli, R. Hemmig, M. Bachmann, and A. MacKenzie. 1998. Increased mature interleukin-1β (IL-1β) secretion from THP-1 cells induced by nigericin is a result of activation of p45 IL-1β-converting enzyme processing. *J. Biol. Chem.* 273: 17846–17851.
71. MacKenzie, A., H. L. Wilson, E. Kiss-Toth, S. K. Dower, R. A. North, and A. Surprenant. 2001. Rapid secretion of interleukin-1β by microvesicle shedding. *Immunity* 15: 825–835.
72. Morel, O., F. Toti, B. Hugel, and J. M. Freyssinet. 2004. Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Curr. Opin. Hematol.* 11: 156–164.
73. Morelli, A., P. Chiozzi, A. Chiesa, D. Ferrari, J. M. Sanz, S. Falzoni, P. Pinton, R. Rizzuto, M. F. Olson, and F. Di Virgilio. 2003. Extracellular ATP causes ROCK I-dependent bleb formation in P2X7-transfected HEK293 cells. *Mol. Biol. Cell* 14: 2655–2664.
74. Bianco, F., E. Pravettoni, A. Colombo, U. Schenk, T. Moller, M. Matteoli, and C. Verderio. 2005. Astrocyte-derived ATP induces vesicle shedding and IL-1β release from microglia. *J. Immunol.* 174: 7268–7277.
75. Pellegatti, P., S. Falzoni, P. Pinton, R. Rizzuto, and F. Di Virgilio. 2005. A novel recombinant plasma membrane-targeted luciferase reveals a new pathway for ATP secretion. *Mol. Biol. Cell* 16: 3659–3665.
76. 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: 579–582.
77. Chakfe, Y., R. Seguin, J. P. Antel, C. Morissette, D. Malo, D. Henderson, and P. Seguela. 2002. ADP and AMP induce interleukin-1β release from microglial cells through activation of ATP-primed P2X$_7$ receptor channels. *J. Neurosci.* 22: 3061–3069.
78. Koshlukova, S. E., T. L. Lloyd, M. W. Araujo, and M. Edgerton. 1999. Salivary histatin 5 induces non-lytic release of ATP from *Candida albicans* leading to cell death. *J. Biol. Chem.* 274: 18872–18879.
79. Bodin, P., and G. Burnstock. 2001. Purinergic signalling: ATP release. *Neurochem. Res.* 26: 959–969.
80. Elssner, A., M. Duncan, M. Gavrilin, and M. D. Wewers. 2004. A novel P2X$_7$ receptor activator, the human cathelicidin-derived peptide LL37, induces IL-1β processing and release. *J. Immunol.* 172: 4987–4994.
81. Crane, J. K., T. M. Naeher, S. S. Choudhari, and E. M. Giroux. 2005. Two pathways for ATP release from host cells in enteropathogenic *Escherichia coli* infection. *Am. J. Physiol.* 289: G407–G417.

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82. Beigi, R., E. Kobatake, M. Aizawa, and G. R. Dubyak. 1999. Detection of local ATP release from activated platelets using cell surface-attached firefly luciferase. *Am. J. Physiol.* 276: C267–C278.
83. Humphreys, B. D., and G. R. Dubyak. 1998. Modulation of $P2X_7$ nucleotide receptor expression by pro- and anti-inflammatory stimuli in THP-1 monocytes. *J. Leukocyte Biol.* 64: 265–273.
84. Narcisse, L., E. Scemes, Y. Zhao, S. C. Lee, and C. F. Brosnan. 2005. The cytokine IL-1β transiently enhances $P2X_7$ receptor expression and function in human astrocytes. *Glia* 49: 245–258.
85. Falzoni, S., M. Munerati, D. Ferrari, S. Spisani, S. Moretti, and F. Di Virgilio. 1995. The purinergic P2Z receptor of human macrophage cells: characterization and possible physiological role. *J. Clin. Invest.* 95: 1207–1216.
86. Derks, R., and K. Beaman. 2004. Regeneration and tolerance factor modulates the effect of adenosine triphosphate-induced interleukin 1β secretion in human macrophages. *Hum. Immunol.* 65: 676–682.
87. Seman, M., S. Adriouch, F. Scheuplein, C. Krebs, D. Freese, G. Glowacki, P. Deterre, F. Haag, and F. Koch-Nolte. 2003. NAD-induced T cell death: ADP-ribosylation of cell surface proteins by ART2 activates the cytolytic $P2X_7$ purinoceptor. *Immunity* 19: 571–582.
88. 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: 2469–2477.
89. Wilson, H. L., S. E. Francis, S. K. Dower, and D. C. Crossman. 2004. Secretion of intracellular IL-1 receptor antagonist (type 1) is dependent on $P2X_7$ receptor activation. *J. Immunol.* 173: 1202–1208.
90. Sluyter, R., J. G. Dalitz, and J. S. Wiley. 2004. $P2X_7$ receptor polymorphism impairs extracellular adenosine 5′-triphosphate-induced interleukin-18 release from human monocytes. *Genes Immun.* 5: 588–591.
91. Hanley, P. J., B. Musset, V. Renigunta, S. H. Limberg, A. H. Dalpke, R. Sus, K. M. Heeg, R. Preisig-Muller, and J. Daut. 2004. Extracellular ATP induces oscillations of intracellular Ca^{2+} and membrane potential and promotes transcription of IL-6 in macrophages. *Proc. Natl. Acad. Sci. USA* 101: 9479–9484.
92. la Sala, A., D. Ferrari, F. Di Virgilio, M. Idzko, J. Norgauer, and G. Girolomoni. 2003. Alerting and tuning the immune response by extracellular nucleotides. *J. Leukocyte Biol.* 73: 339–343.
93. Nathan, C. 2002. Points of control in inflammation. *Nature* 420: 846–852.
94. Cheewatrakoolpong, B., H. Gilchrest, J. C. Anthes, and S. Greenfeder. 2005. Identification and characterization of splice variants of the human $P2X_7$ ATP channel. *Biochem. Biophys. Res. Commun.* 332: 17–27.

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