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Extracellular ATP May Contribute to Tissue Repair by Rapidly Stimulating Purinergic Receptor X7-Dependent Vascular Endothelial Growth Factor Release from Primary Human Monocytes

Lindsay M. Hill,* Monica L. Gavala,† Lisa Y. Lenertz, † and Paul J. Bertics†

Extracellular ATP has been proposed to act as a danger signal to alert the immune system of cell damage. Release of high local concentrations of ATP activates the nucleotide receptor, purinergic receptor X7 (P2RX7), on monocytic cells, which promotes the processing/release of proinflammatory mediators. Although the proinflammatory actions of P2RX7 are well recognized, little is known regarding the potential function of P2RX7 in repair responses. Because the resolution of inflammation is characterized by monocytic cell-dependent production of proangiogenic factors, we evaluated the contribution of P2RX7 to this process. We observed that both short-term and long-term P2RX7 activation promotes the robust release of vascular endothelial growth factor from primary human monocytes. This vascular endothelial growth factor release is calcium dependent and associated with reactive oxygen species production. This previously unrecognized action of P2RX7 suggests that it may not only participate in inflammation and cell death, but that it is also likely to be important in the control of angiogenesis and wound repair. The Journal of Immunology, 2010, 185: 3028–3034.

Damage-associated molecular patterns are associated with molecules that are produced and/or released by cells undergoing stress or death. These agents often act as costimulatory signals that initiate a highly regulated immune response (1). Extracellular ATP can act as a damage-associated molecular pattern, given that it is normally confined to intracellular sites, but can be released at high local levels following cell lysis, infection, or via regulated efflux (2, 3).

ATP released into the extracellular space can modulate the immune response through their capacity to bind and activate multiple nucleotide receptor family members. Of relevance to this study, the nucleotide receptor, purinergic receptor X7 (P2RX7), can function as a ligand-gated ion channel and has been implicated in the progression of several inflammatory disorders, including sepsis, arthritis, and tuberculosis (4–6). The activation of P2RX7 on monocytic cells, which play key roles in all phases of inflammation, is known to initiate or enhance the production and release of several proinflammatory mediators, including IL-1β, TNF-α, inducible NO synthase, and reactive oxygen species (ROS) (7–11).

In addition to the production of proinflammatory mediators that promote the progression of inflammation, monocytic cells also have a major role in inflammatory wound repair via their ability to scavenger cellular debris and to produce proangiogenic factors. A key angiogenic factor produced by activated monocytic cells is vascular endothelial growth factor (VEGF) (12, 13). VEGF promotes new blood vessel formation, induces endothelial cell proliferation, and initiates immune cell migration (14, 15). Interestingly, VEGF release in monocytic cells is often induced by shear stress and hypoxia (15, 16), which are previously recognized mechanisms of ATP release in several cell types (17, 18). VEGF is important in many pathological processes, including tumor progression, arthritis, and ischemia (15, 19, 20). Although the proinflammatory role of P2RX7 in monocytic cell function is well documented, little is known about P2RX7 action in monocytic-dependent wound repair, and no reports have linked P2RX7 to the production of angiogenic proteins in these cells.

Because P2RX7 action is known to potentiate inflammatory mechanisms that can promote tissue damage, it would be a potentially important homeostatic mechanism if this receptor system also initiated effects that would ultimately facilitate the repair of damage induced during the inflammatory response. In this regard, activated monocytic cells serve a key action by initiating angiogenesis and tissue repair, and thus, we tested the hypothesis that activation of P2RX7 on monocytes would result in the production and release of the proangiogenic and wound repair-associated factor VEGF. In the current study, we report a previously unrecognized action of P2RX7 agonists, namely the stimulation of the robust, concentration- and time-dependent expression, and release of VEGF from primary human monocytes. The expression and release of VEGF were found to be P2RX7 dependent, and the release of this proangiogenic factor is linked to both intracellular calcium (Ca²⁺) influxes and ROS production. This process reveals that P2RX7 may not only function in inflammation and cell death, but that it may also participate in the control of angiogenesis and wound repair.

Materials and Methods

Materials

ATP, 2(3′,5′)-O-(4-benzoylbenzoyl)-ATP (BrzATP), 2-methylthioadenosine 5′-monophosphate (MeSAMP), PMA, LPS (Escherichia coli, serotype O111:...
0111B4), EGTA, and N-acetyl cysteine (NAC) were purchased from Sigma-Aldrich (St. Louis, MO). BAPTA-AM was purchased from Invitrogen (Carlsbad, CA). The P2RX7 antagonist A438079 and P2Y11 antagonist NF-157 were acquired from Tocris (Ellisville, MO).

**Isolation of human blood monocytes**

Human blood-derived monocytes were purified, as described (21). Heparinized blood was drawn from healthy donor volunteers at the University of Wisconsin Hospital in compliance with an approved human subjects protocol, and cells were separated using a Percoll density gradient. CD14+ cells were identified by flow cytometry and comprised 90–95% of the population. Cells were maintained at 37°C in a humidified atmosphere with 5% CO2 in RPMI 1640 supplemented with 10% FBS (HyClone, Logan, UT), 2 mM sodium pyruvate, 2 mM l-glutamine, and 100 U/ml penicillin/streptomycin.

ELISA

A sandwich ELISA for human VEGF was performed using Abs obtained from R&D Systems (Minneapolis, MN), according to the manufacturer’s protocol. VEGF concentrations were calculated via interpolation from a standard curve, and all determinations were performed in duplicate.

**Quantitative RT-PCR**

Primers used were directed toward human VEGF or 18S (loading control) and are as follows: VEGF primer, 5’-ATCTTCAAGCCTATCTGTTGC-GC-3’, R 5’-GCTGACACCTGAGCTGGTTG-3’; 18S primer, 5’-GGACACCGGAACAGATGACAG-3’, R 5’-ATGCCTACCACACCTAGAACAGG-3’. PCR reactions were detected by SYBR Green iQ supermix dye (Bio-Rad, Hercules, CA) and were performed on an I-cycler real-time thermal cycler (Bio-Rad; 54°C annealing temperature, 50 cycles).

**Cell cytotoxicity assays**

Human monocytes were plated in 96-well plates at a density of 1 x 10^5 cells/ml. In certain cases, the cells were pretreated with either BAPTA-AM or P2RX7 antagonist A438079, followed by stimulation with BzATP or ATP for 4 h at 37°C. Following these incubations, cell viability was assessed by either using the nonradioactive cell proliferation assay (3-4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt [MTS]-based protocol. VEGF concentrations were calculated via interpolation from a standard curve, and all determinations were performed in duplicate.

**Results**

**VEGF release following stimulation of human monocytes with P2RX7 ligands**

Primary human monocytes were stimulated for 24 h with the P2RX7 agonists 100 μM BzATP or 300 μM ATP, as well as with a known stimulus for VEGF release, that is, LPS (1 μg/ml) (22). The cell supernatants were then assayed for VEGF release. When compared with vehicle control, the data in Fig. 1A demonstrate a >6-fold increase in the release of VEGF from monocyteic cells after stimulation with BzATP for 24 h and a >3-fold increase in VEGF release after ATP stimulation for 24 h. A detailed evaluation of P2RX7 agonist-induced effects revealed that the VEGF release is both time and concentration dependent. When cells were treated with 100 μM BzATP or 1 μg/ml LPS for 1–24 h, we observed that the P2RX7 agonist BzATP promotes a statistically significant release of VEGF in as little as 1 h (Fig. 1B). Conversely, the levels of VEGF release induced by high concentrations of LPS are not significant until 16 h posttreatment.

**Short-term stimulation by P2RX7 agonists induces VEGF release from human monocytes**

Recent work has shown that short-term activation of P2RX7 (<1 h) can result in altered gene expression (8). To evaluate whether short-term stimulation with P2RX7 agonists can also induce VEGF release, primary cells were treated for 5 min with 100 μM BzATP, 300 μM ATP, or 1 μg/ml PMA [a known inducer of VEGF release (23)]. The media was then removed and replaced with fresh media containing no agonists. Supernatants were harvested after 4 h and assayed for VEGF release. As shown in Fig. 2A, short-term exposure to BzATP and ATP results in significant VEGF release (2- and 4-fold, respectively). The data in Fig. 2B depict the results of short-term BzATP-induced VEGF release as pg/ml for each individual patient.

**A P2RX7-selective antagonist attenuates BzATP- and ATP-induced VEGF release**

Although BzATP is a potent agonist of P2RX7, it can stimulate a few other P2 receptors (e.g., P2RY11–13) at high concentrations. To examine the specific involvement of P2RX7 in regulating VEGF release, we evaluated the sensitivity of nucleotide-induced VEGF release to the addition of the selective P2RX7 antagonist A438079. This antagonist has been previously shown to have no detectable effects on other P2X and P2Y receptors (24). The results in Fig. 3A reveal that the release of VEGF by primary cells treated for 4 h with either 100 μM BzATP or 300 μM ATP, but not with PMA, is concentration dependently inhibited by the P2RX7 antagonist A438079. Data in Supplemental Fig. 1 demonstrate VEGF release.

![FIGURE 1. P2RX7 agonists stimulate the time- and concentration-dependent release of VEGF from primary human monocytes. A, Primary human monocytes purified from healthy volunteer donors were treated with control (HEPES), 100 μM BzATP, 300 μM ATP, or 1 μg/ml LPS for 24 h. B, Cells were treated with either control, 100 μM BzATP, or 1 μg/ml LPS for 1–24 h. C, Cells were treated with control or varying amounts of the P2RX7 agonist BzATP for 4 h. The results depicted in A–C each represent at least three independent experiments, with scale bars representing mean ± SEM. D, Cells were treated with control or varying amounts of the P2RX7 agonist ATP for 4 h. Single gray data points represent VEGF release (pg/ml) from each donor after 100 μM BzATP treatment. The results depicted in D are from three independent donors, with scale bars representing mean ± range, and numbers in parentheses indicating fold increase in VEGF release after treatment with 500 μM ATP as compared with control for each donor. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001.](http://www.jimmunol.org/Downloadedfrom)
(pg/ml) following pretreatment with A438079 for each individual donor. The percentage of inhibition of BzATP-induced VEGF after preincubation with the P2RX7 antagonist was observed to be similar following 24-h treatment (our unpublished observations). To ensure that A438079 pretreatment was not attenuating P2RX7 agonist-induced VEGF release because of cytotoxicity, MTS viability assays were performed in parallel with the VEGF release experiments. As illustrated in Fig. 3B, pretreatment with A438079 had no significant effect on the metabolic activity of the cells.

To assess the contribution of other P2 receptors to VEGF release, selective antagonists for P2RY11 and P2RY12/13 were used. The results in Fig. 3C and 3D show that preincubation of primary human monocyctic cells with the P2RY11 antagonist NF-157, or the P2RY12/13 antagonist MeSAMP, had no effect on BzATP-, ATP-, or PMA-induced VEGF release. Also, the coaddition of NF-157 or MeSAMP with the P2RX7 antagonist A438079 resulted in a similar degree of attenuation of BzATP-induced VEGF as the addition of the P2RX7 antagonist alone (our unpublished observations). However, as seen previously, we found that NF-157 and MeSAMP could attenuate the P2Y11-dependent IL-8 release (25) and the P2Y12/13-dependent stimulation of ERK phosphorylation (26), respectively (Supplemental Figs. 2, 3). These data support the hypothesis that BzATP- and ATP-induced VEGF release is primarily associated with P2RX7 activation in human monocytic cells.

**BzATP and ATP induce P2RX7-dependent VEGF expression**

To determine whether P2RX7 agonists induce VEGF expression as well as release, we evaluated the effect of BzATP and ATP on relative mRNA levels of VEGF via quantitative RT-PCR. Primary human monocytes were stimulated with the P2RX7 agonists 100 μM BzATP, 300 μM ATP, or 1 μg/ml PMA for 0.5 and 1 h, and cell lysates were subsequently analyzed for VEGF mRNA by RT-PCR, as outlined in Materials and Methods. The results presented in Fig. 4A indicate that P2RX7 agonists can induce a robust, time-dependent increase in VEGF expression in a little
as 1 h of treatment time. To evaluate whether induction of VEGF expression by BzATP and ATP is dependent on P2RX7 activation, we determined the sensitivity of nucleotide-induced VEGF expression to the addition of the selective P2RX7 antagonist A438079. As demonstrated in Fig. 4B, preincubation with the P2RX7 antagonist A438079 attenuates BzATP-induced VEGF expression in a concentration- and time-dependent manner. These results further support the idea that nucleotide-induced VEGF expression is dependent upon P2RX7 activation.

LPS priming or costimulation does not enhance P2RX7 agonist-induced VEGF release

Several P2RX7 signaling events are enhanced by priming or the coadministration of various immune stimuli, such as LPS (7, 8). To determine whether LPS priming could modulate nucleotide-induced VEGF release, primary human monocytes were first treated with 100 ng/ml LPS for 4 h and then stimulated with BzATP for 2 h. Fig. 5A provides data demonstrating that LPS priming has no detectable effect on BzATP-induced VEGF release. To examine whether LPS coadministration can alter BzATP-induced VEGF release, monocytes were stimulated with 100 μM BzATP and various concentrations of LPS for 4 and 24 h. The results shown in Fig. 5B and 5C indicate that coadministration with LPS does not significantly enhance BzATP-induced VEGF release at 4 or 24 h. These results indicate that P2RX7 agonist-induced VEGF release is not dependent on, nor enhanced by, priming or coadministration with LPS.

P2RX7 agonist-induced VEGF release is Ca2+ dependent

To determine whether the P2RX7 agonist-stimulated release of VEGF by primary human monocytes is linked to the rapid increase in intracellular Ca2+ observed after P2RX7 ligation, we examined the influence of treatment with EGTA (an extracellular Ca2+ chelator) or a cell-permeable Ca2+ chelator BAPTA-AM on this process. Cells were incubated with BAPTA-AM (3–5 μM) or EGTA (1–3 mM) for 20 min prior to stimulation with 100 μM BzATP, 300 μM ATP, or the Ca2+ ionophore ionomycin (1 μg/ml). As shown in Fig. 6A, pretreatment of monocytic cells with BAPTA-AM significantly attenuated P2RX7 agonist-induced VEGF release. Conversely, ionomycin treatment alone was unable to stimulate robust VEGF release from these cells (VEGF levels: 131 ± 76 pg/ml [ionomycin treated] compared with 153 ± 72 pg/ml [vehicle treated]). Similarly, as shown in Fig. 6C, pretreatment with EGTA concentration dependently attenuated P2RX7 agonist-induced VEGF release. To ensure that BAPTA-AM or EGTA pretreatment was not attenuating P2RX7 agonist-induced VEGF release because of its possible cytotoxic effects, we performed MTS viability assays in parallel with the VEGF release experiments. As shown in Fig. 6B and 6D, respectively, pretreatment with BAPTA-AM or EGTA has no significant effect on the metabolic activity of the cells. Collectively, these results provide evidence that Ca2+ fluxes are necessary, but not sufficient, for P2RX7 agonist-induced VEGF release.

P2RX7 agonist-induced VEGF release is sensitive to the antioxidant NAC

Although P2RX7 agonist-induced VEGF release appears dependent on an increase in intracellular Ca2+ levels, the failure of ionomycin to trigger VEGF release suggests that an elevation of cytoplasmic Ca2+ is not sufficient for this process. Because VEGF is known to be induced by shear stress and hypoxia, and given that activation of P2RX7 can induce the rapid production of ROS in a Ca2+-independent manner (8, 27), we tested the hypothesis that P2RX7

**FIGURE 5.** LPS priming or costimulation does not synergistically enhance P2RX7 agonist-induced VEGF production. A, Primary human monocytes were primed with vehicle control (HEPES) or 100 ng/ml LPS for 4 h and stimulated with either vehicle control (HEPES) or 100 μM BzATP for 2 h. B and C, Primary human monocytes were stimulated with vehicle control (HEPES), 100 μM BzATP, 0.01 μg/ml LPS, 0.1 μg/ml LPS, or costimulated with 100 μM BzATP/0.01 μg/ml LPS or 100 μM BzATP/0.1 μg/ml LPS for 4 h (B) or 24 h (C). Supernatants were collected and analyzed for VEGF release. The results depicted in A–C each represent three independent experiments (mean ± SEM).
agonist-induced VEGF release in primary human monocytes is linked, at least in part, to ROS production. In these experiments, cells were pretreated with the antioxidant NAC (10 mM) for 20 min prior to stimulation with 100 μM BzATP or 300 μM ATP. Fig. 7 illustrates that NAC pretreatment attenuates P2RX7 agonist-induced VEGF release. Conversely, hydrogen peroxide (H₂O₂) treatment alone (169 ± 70 pg/ml, compared with control, 153 ± 72 pg/ml), or in combination with ionomycin treatment (170 ± 85 pg/ml, compared with control, 153 ± 72 pg/ml), is unable to stimulate robust VEGF release from these cells. Taken together, these results indicate that P2RX7-mediated VEGF release is dependent on both Ca²⁺ and ROS production, but that additional processes are likely to be critical.

Discussion
Monocytic cells play an integral role in the resolution of inflammation via the production of angiogenic factors that stimulate new blood vessel formation and allow for immune cell trafficking, tissue remodeling, and repair. Relatively little is understood regarding the role of P2RX7 in monocyte cell-dependent wound repair, and there are no previous reports demonstrating a link between P2RX7 activation and the production of angiogenic proteins in these cells. In this study, we provide evidence supporting a previously unrecognized action of P2RX7 signaling, namely the capacity to induce rapid and robust expression and release of the proangiogenic factor VEGF from primary human monocytes. The magnitude of VEGF release after stimulation with P2RX7 agonists appears biologically relevant in that it is comparable to the concentrations of this factor found in the serum of patients after injury or infection (28, 29) and comparable to that induced by LPS.

Although BzATP is a selective agonist for P2RX7, it can activate other P2 receptors at high concentrations. The data presented in this work indicate that VEGF release stimulated by P2RX7 agonists is concentration dependently attenuated by the selective P2RX7 antagonist A438079, whereas PMA-stimulated VEGF release is unaltered by the addition of the P2RX7 antagonist. This attenuation does not appear to arise from possible nonspecific cytotoxic effects, as two independent viability assays were performed in parallel and both indicated that the P2RX7 antagonist had no discernible effect on cell viability (Fig. 3B, Supplemental Fig. 4). This antagonist can block several BzATP-induced P2RX7 events and appears devoid of activity toward other P2 receptors and a wide array of other ion channels (24). To assess the contribution of other P2 receptors to VEGF release, we used several selective agonists and antagonists. We found that uridine nucleotides UTP and UDP were unable to stimulate substantial VEGF release (79 ± 3 pg/ml and 84 ± 2 pg/ml, respectively, compared with control, 60 ± 5 pg/ml), suggesting that P2 receptors sensitive to these nucleotides, such as P2RY2, P2RY4, and P2RY6, are not likely to be significantly involved in nucleotide-induced VEGF release (our unpublished observation). Also, selective inhibition of P2RY1, P2RY12, or P2RY13, which are receptors that can be activated by high concentrations of BzATP, had no detectable effect on nucleotide-induced VEGF release. Together, these data are consistent with the idea that BzATP- and ATP-induced VEGF release is largely dependent on P2RX7 signaling.

As discussed above, the rapid production and release of VEGF by primary monocytes are selectively observed with P2RX7 agonists, whereas LPS-stimulated VEGF release requires a considerably longer period of time to become detectable by ELISA. This difference in the time course of action suggests several possibilities. For example, differing signaling mechanisms of VEGF induction may exist between P2RX7- and LPS-dependent systems. In this regard, VEGF production can be regulated at the level of transcription, mRNA stability, and translation, thereby allowing for variations in the kinetics of VEGF production in response to distinct stimuli (30). We were able to determine that P2RX7 agonists induce robust, A438079-sensitive expression of VEGF mRNA (40- to 70-fold after 1 h) in primary human monocytes, in contrast to the modest levels seen after nucleotide stimulation of a glioma cell line (0.6-fold after 8 h) (31). These observations support the idea that P2RX7 nucleotides are able to affect VEGF expression at the transcriptional level and provide further evidence for the role of P2RX7 in this modulation. In addition, because several cell types have been reported to release ATP following LPS administration (32, 33), the differing time course of action between P2RX7 ligands and LPS may be attributable to a delayed autocrine effect initiated by LPS that partly involves P2RX7 signaling events. However, this possibility appears less likely because we observed that the P2RX7 antagonist A438079 did not attenuate LPS-stimulated VEGF release (our unpublished observation). Interestingly, although P2RX7 activation is known to enhance several signaling processes initiated by other immune stimulants, such as LPS (7, 8), it is noteworthy that LPS priming of primary human monocytes did not enhance P2RX7 agonist-induced VEGF, and costimulation with P2RX7 agonists and LPS did not result in synergistic enhancement of VEGF release. Thus, P2RX7 agonist-induced VEGF appears unique in that its release is not dependent on coinadministration with other immune stimulants.

Another consideration with respect to the dynamics of VEGF release is that short-term activation of P2RX7 may more accurately recapitulate the transient availability of ATP after events such as cell lysis at sites of injury or infection. In this respect, data presented in this work indicate that activation of P2RX7 for as little as 5 min results in significant VEGF release from primary human monocytes. Therefore, the release of VEGF after short-term stimulation of P2RX7 may be relevant in multiple injury models, including ischemic stroke, in which the presence of ATP in the extracellular environment is markedly, but transiently elevated.

The activation of P2RX7 by high concentrations of ATP observed during inflammation is known to result in cell death in as little as 4–5 h. Interestingly, our results reveal significant VEGF release after cell stimulation with very low levels of P2RX7 agonists. Few P2RX7-dependent signaling pathways are known to be sensitive to such small concentrations of agonist, suggesting that VEGF release is an exquisitely sensitive endpoint for P2RX7 action. It is of note that these low concentrations of BzATP and ATP do not result in cell death even 24 h posttreatment (Supplemental Fig. 5), suggesting a role for P2RX7-dependent VEGF release in the absence of nucleotide-induced apoptosis or necrosis.

**FIGURE 7.** P2RX7 agonist-induced VEGF production is sensitive to NAC. Primary human monocytes were pretreated with either vehicle control (HEPES) or 10 mM NAC for 20 min at 37°C. Following preincubation, cells were treated with control, 100 μM BzATP, or 300 μM ATP for 4 h. Supernatants were collected and assayed for VEGF. The results represent three independent experiments (mean ± SEM). *p < 0.05; **p < 0.01.
Recently, several papers have identified P2RX7-dependent protein expression in the absence of cell death (8, 21, 32, 34), supporting the idea that P2RX7 may be capable of participating in more long-term responses, such as wound repair and angiogenesis. Because VEGF can stimulate several transcription factors recently linked to P2RX7 activation, namely Egr-1 and AP-1 (33, 35), it is conceivable that P2RX7-induced VEGF may also play a role in modulating P2RX7-dependent gene expression.

P2RX7 activation results in a rapid influx of Ca\textsuperscript{2+} ions, and our data support a role for an elevation of intracellular-free Ca\textsuperscript{2+} in P2RX7-induced VEGF release. However, ionomycin alone is unable to stimulate VEGF release, suggesting that an elevation of cytoplasmic Ca\textsuperscript{2+} is necessary, but not sufficient, for VEGF release. Although P2RX7 is a ligand-gated ion channel, it does signal via several Ca\textsuperscript{2+}-independent mechanisms, including ROS production (8, 27), which in turn can stimulate the MAPKs p38 and JNK (7). We observed that P2RX7 agonist-induced VEGF release is sensitive to the antioxidant NAC, supporting a role for ROS in VEGF release. However, H\textsubscript{2}O\textsubscript{2} stimulation alone, or in concert with ionomycin, is insufficient to produce VEGF release from primary cells. These data suggest that multiple P2RX7-dependent signaling events must co-occur to enable robust VEGF release in these cells, and that Ca\textsuperscript{2+} influxes together with ROS production cannot solely recapitulate this P2RX7-dependent event. It is noteworthy that inhibition of the MAPKs ERK, p38, and JNK, which are Ca\textsuperscript{2+}- and/or ROS-sensitive P2RX7 end-points, has no discernible effect on P2RX7 agonist-induced VEGF release (our unpublished observation). Altogether, our data suggest that a Ca\textsuperscript{2+}- and ROS-independent effecter(s) upstream of VEGF release can contribute to P2RX7 signaling.

Nucleotide release can occur by processes other than infection and cytolsis, including platelet degranulation and release through certain membrane channels (36). Endothelial cells are known to release ATP using nonlytic pathways in response to shear stress or hypoxia (37, 38). Therefore, endothelial cells may activate P2RX7 on circulating monocytes and trigger VEGF production as a means to combat hypoxic environments resulting from normal endothelial turnover. ATP release and subsequent P2RX7 activation have also been implicated in cell transformation, and P2RX7 has been linked to numerous cancers, including chronic lymphocytic leukemia, neuroblastomas, and cervix squamous carcinoma (for review, see Ref. 8). Disregulated VEGF production is often a hallmark of tumor development and linked to tumor angiogenesis (19). Thus, ATP-activated monocytes within a tumor environment may contribute to tumor vascularization and growth through P2RX7-dependent VEGF release. The targeting of P2RX7 or P2RX7-dependent VEGF release may prove beneficial in the treatment of tumor growth.

In sum, P2RX7 activation can promote the rapid and robust release of VEGF from primary human monocytes, suggesting a previously unrecognized role for P2RX7 in angiogenesis and wound repair.

Disclosures

The authors have no financial conflicts of interest.

References


Supplemental Figure Legends

Supplemental Figure 1: **P2RX7 antagonist A438079 attenuates BzATP and ATP-induced VEGF production in donors.** (A) Primary human monocytes from five independent patients were pre-incubated with vehicle or A438079 (3 µM or 10 µM) for 30 min at 37°C. The cells were then treated with control (HEPES) or 100 µM BzATP and supernatants were assayed for VEGF. The data are the same as that shown in Fig 3A but are plotted as VEGF Release (pg/mL) for each individual donor.

Supplemental Figure 2: **P2RY11 antagonist inhibits ATPyS-induced IL-8 release in primary human monocytes.** Primary human monocytes were pre-incubated with vehicle or P2RY11 antagonist NF 157 (1 µM) for 30 min at 37°C. The cells were then treated with control (HEPES), 100 µM BzATP, or 300 µM ATPγS for 24 h and supernatants were assayed for hIL-8. Data from a representative experiment are shown as averages with error bars representing mean +/- SD. Results are representative of at least 2 comparable experiments.

Supplemental Figure 3: **P2RY12/13 antagonist inhibits ADP-induced ERK1/2 phosphorylation.** Primary human monocytes were pre-incubated with vehicle or P2RY12/13 antagonist 2-Methylthioadenosine 5’-monophosphate (MeSAMP) (3 µM) for 30 min at 37°C. The cells were then treated with control (HEPES) or 300 µM ADP for 30 min and lysates were assayed for ERK1/2 phosphorylation. Data from a representative experiment are shown and the results are representative of at least 2 comparable experiments.
**Supplemental Figure 4:** Primary monocyte viability results from MTS and Trypan Blue exclusion after pretreatment with P2RX7 antagonist and stimulation with P2RX7 agonists. Primary human monocytes were pre-incubated with vehicle or A438079 (3 µM or 10 µM) for 30 min at 37°C. The cells were then treated with control (HEPES), 100 µM BzATP, or 300 µM ATP for 4 h. Cell viability was assessed through MTS (A) or Trypan Blue exclusion (B) and data from a representative experiment are shown as averages with error bars representing mean +/- SD. Results are representative of at least 2 comparable experiments.

**Supplemental Figure 5:** Low concentrations of P2RX7 agonists do not result in cell death. Primary human monocytes were stimulated with either vehicle control (HEPES), 100 µM BzATP, or 300 µM ATP for 24 h. The MTS reagent was then added to assess cell viability and absorbance was read as described in materials and methods. Results depicted represent at least three independent experiments (3 distinct donors) and were summarized and graphed with error bars illustrating +/- SEM.