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Trends in Pharmacological Sciences

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Opinion

Purinergic Signaling: A New Pharmacological Target Against Viruses?

Davide Ferrari,[1,*] Marco Idzko,[2] Tobias Müller,[3] Roberto Manservigi,[1] and Peggy Marconi[4]

Viral diseases represent a major global problem in human health, with high morbidity and mortality. Despite recent progress in antiviral treatments, several viral diseases are still not controlled and millions suffer from them every year. It has recently emerged that purinergic signaling participates in viral infection and replication. Furthermore, stimulation of purinergic receptors in infected cells also induces inflammatory and antiviral responses, thus contributing to the host antiviral defense. Here we review the multiple roles played by the purinergic signaling network in cell–virus interactions that can lead either to viral maintenance in the cells or, by contrast, to stronger antiviral responses, and discuss potential future applications of purinergic signaling modulation for the treatment of viral diseases.

Highlights

Viral infection induces the release of intracellular nucleotides (ATP, UTP) into the extracellular fluid.

Autocrine activation of purinergic receptors expressed by host cells promotes viral entry and the replication of some viruses. Accordingly, inhibition of purinergic receptors impairs viral infection and the production of viral particles.

Pharmacological stimulation of eukaryotic cells with ATP or massive nucleotide release due to extensive cell damage impairs cell–virus interaction and stimulates antiviral responses such as chemotaxis, transcription of antiviral cytokine genes, and the release of inflammatory mediators.

Modulation of purinergic receptors expressed by immune cells might reduce the inflammatory responses and exacerbation of disease symptoms occurring as a consequence of the viral infection, such as in the case of asthma, cardiac inflammation, and neurological impairment, thus ameliorating the patient's condition.

Targeting of purinergic signaling may represent a new possibility to control viral infections and the pathological consequences of viral diseases.

Modulating Cell Functions from Outside via Purinergic Signaling

Intracellular adenosine (ATP, ADP) and uridine (UTP, UDP) nucleotides and the nucleoside adenosine (ADO) play roles as enzymatic modulators, fundamental molecular building blocks for nucleic acids, and energy providers to the cell [1,2]. However, the formation or accumulation of nucleotides and nucleosides can also occur extracellularly, where they are transported, released, formed by hydrolysis, or even synthesized by enzymes expressed on the outer side of the plasma membrane [3]. Immune cells, neuronal cells, thrombocytes, and endothelial cells are known to release nucleotides, particularly ATP, into the extracellular space. This in turn is essential for neuronal transmission, cardiac contraction, endothelial relaxation, coagulation, and immune responses [4–6].

Many biological stressors, such as bacteria, protozoa, parasites, and viruses, are endowed with the ability to induce ATP release from the eukaryotic cell. This release is interpreted as an alarm signal from damaged cells/tissues to the immune cells to start/amplify defensive responses. Extracellular ATP [7,8] stimulates inflammatory responses against the microbes and enables phagocytes to localize and engulf dying cells [9–12]. However, this can also result in excessive stimulation of the immune cells giving rise to chronic inflammatory disorders such as asthma, contact hypersensitivity, Crohn's disease, and graft-versus-host disease [13] or to acute systemic inflammatory response such as septic shock [14].

ATP concentration is high in the cytoplasm and low in the extracellular compartment (1–10 mM vs 10–100 nM, respectively) [15,16]. The extracellular ATP concentration is kept low by the activity of nucleotide hydrolyzing enzymes expressed on the cell membrane where ATP and ADP are transformed to AMP by CD39, an ectonucleoside triphosphate diphosphohydrolase, and subsequently to ADO by the ecto-5′-nucleotidase CD73 [17,18]. Extracellular ADO is then transported into the cells by equilibrative nucleoside transporter 1 and 2 (ENT1 and ENT2) or inactivated at the plasma membrane to inosine by adenosine deaminase (ADA)/CD26 (Figure 1). ATP and other nucleotides (ADP, UTP, and UDP) can be sensed by a specific subtype of plasma membrane receptors known as purinergic 2 (P2) receptors, which are further classified

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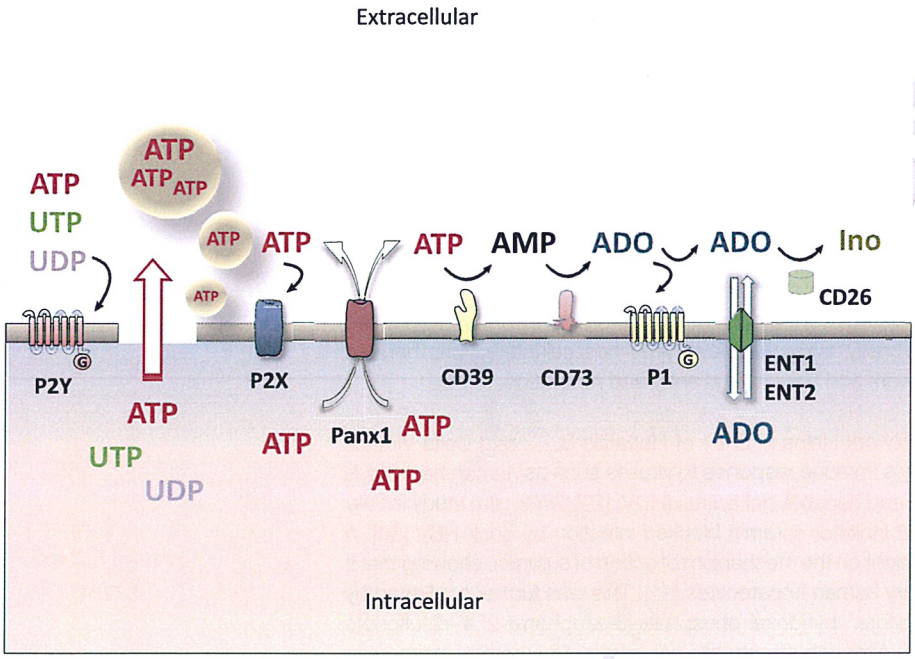

Q7 Figure 1. Extracellular Purinergic Signaling. ATP, ADP, UTP, UDP, and adenosine (ADO) are synthesized intracellularly. These are released by exocytosis, specialized plasma membrane proteins (Panx1, P2X7), plasma membrane vesicles, and membrane stress/damage. They act as signaling molecules by activating purinergic P2 (P2X and P2Y) and P1 (A_1, A_{2A}, A_{2B}, A_3) receptors. ATP and ADP are also hydrolyzed by the ectonucleotidases CD39 and CD73, generating the P1 receptor agonist ADO, which can be transported into the cytoplasm by the ENT1 and ENT2 proteins or degraded extracellularly to inosine (Ino) by adenosine deaminase (ADA/CD26).

as ionotropic (P2X1–7) or metabotropic (P2Y1, 2, 4, 6, 11, 12, 13, 14) [19,20] based on their sequence, membrane topology, and agonist selectivity. P2X receptors are activated by extracellular ATP [20] while P2Y subtypes are seven-membrane-spanning G protein-coupled receptors activated by the nucleotides ATP, ADP, UTP, and UDP. While ATP is an agonist for P2Y1, P2Y2, and P2Y11, UTP and UDP activate the P2Y2, P2Y4, and P2Y6 receptors and the nucleotide sugar UDP-glucose is an agonist for P2Y14 [21]. The other class of purinergic receptors, the metabotropic G protein-coupled P1 receptors, are activated by extracellular ADO. The P1 receptors are further classified into four subtypes: A_1, A_{2A}, A_{2B}, and A_3 [21].

The complexity of the purinergic network seems to be needed for the appropriate balance between the synthetic and catabolic nucleotide pathways to avoid overstimulation of the purinergic receptor network that can lead to excessive immune response and tissue damage [22]. In this perspective, we discuss examples of virus–host cell interactions that modulate the purinergic network leading either to proviral responses including viral maintenance and replication inside host cells or, by contrast, to strong antiviral responses that could potentially be therapeutic against viral infections.

Viral Modulation of the Purinergic Network towards Proviral Responses

A link between extracellular nucleotides/nucleosides and viruses has been neglected for years despite early observations identifying extracellular ATPase-like activity in HeLa cells infected by

herpes simplex virus (HSV) and on the surface of two types of myxovirus [23,24]. In 1968, the presence of ADO in extracellular fluid was shown to affect viral biology. It was further demonstrated that ADO reduced the yield of vaccinia virus by activating A_2 receptors and protein kinase A (PKA) [25,26]. Furthermore, expression of CD39 has been found to be increased on the plasma membrane of lymphocytes from HIV-1-positive patients [27].

The purinergic signaling network participates in various ways in the modulation of the inflammatory response to viral infection. For example, adenosinergic A_{2A} receptors partially contribute to complex, long-lasting protection against neurological immune-mediated damage in a Theiler's murine encephalomyelitis virus (TMEV) model of multiple sclerosis, suggesting that inhibitors of P1 receptors might be an option for the treatment of the inflammatory component of the virus-induced neurological damage [28]. Below we briefly review the known virus–host cell interactions that lead to modulation of the purinergic network and help in virus entry and replication.

Virus-Mediated Activation of P2 Network Helps in Entry of Hepatitis B, C, and Delta Viruses

Viral hepatitis is caused by the body's immune response to viruses such as human hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis delta virus (HDV) [29]. An *in vitro* study initially showed that the large-spectrum P2 inhibitor suramin blocked infection by duck HBV [30]. A more recent report has shed further light on the mechanism of action of suramin, showing that it interferes with HDV entry into primary human hepatocytes [31]. This was further confirmed by experiments with the P2 inhibitors pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate (PPADS) and brilliant blue G (BBG). Although structurally not related to suramin, these were able to inhibit HBV and HDV infection. These observations strongly suggest that at least some P2 receptors, putatively P2X receptors, are crucial for viral entry into hepatocytes [31]. Another interesting aspect of the involvement of purinergic receptors in viral modification of cell physiology was recently shown in HBV-related hepatocellular carcinoma [32]. In HBV-related hepatocellular carcinoma, protein kinase B or Akt is upregulated by HBV. It was observed that atorvastatin, a member of the statin family, decreased HBV X (HBx) protein, insulin-induced pAkt, and pGsk3β (Ser9), levels, which was largely dependent on P2X receptors [32]. Since statins also decrease the proliferation rate and invasiveness of virus-transformed hepatocytes, it would be extremely important to investigate the participation of P2 receptors in blocking these functions in detail, towards therapies designed for hepatocellular carcinoma.

Work on the human hepatoma cell line Huh-7 stably expressing the HCV envelope proteins showed a sixfold increase in P2X4 mRNA and downregulation of P2X5 mRNA compared with control cells [33]. However, it is not yet established whether modification of P2X mRNA expression is also paralleled by substantial changes in receptor protein levels. It was further reported that P2X1 and P2X7 mRNA were upregulated by 2.2 and 2.5 times, respectively, in peripheral blood mononuclear cells (PBMCs) isolated from treatment-naïve chronic HCV patients (i.e., subjects having no pharmacological treatment) compared with healthy controls. Peculiarly, gene expression of the P2X7 subtype was higher in patients responding to the antiviral treatment while it was found to be unchanged compared with healthy controls in those not responding to the therapy [34]. Although these observations are potentially interesting, one cannot exclude that upregulation of the P2X7 mRNA might have been induced by therapeutic administration of interferon to the patients. The functional aspect of virus-induced modulation of P2X mRNA remains to be determined [35].

Virus-Mediated Activation of P2 Network Helps in Entry of HIV

HIV is the etiological agent of AIDS, a slowly evolving disease giving rise to chronic infections that if not treated have a fatal outcome [36]. The HIV-1 envelope glycoprotein complex (Env), a

160-kDa protein precursor, is cleaved and processed into the glycoproteins gp120 and gp41, which mediates fusion of the viral particle to the host T cell via CD4 and the chemokine receptors CXCR4 or CCR5, which acts as coreceptors (Figure 2). An interesting finding is that Env induces ATP release from HIV-1-infected cells within the first 2 h of infection [37]. Moreover, the presence of the ATP-hydrolyzing enzyme apyrase dose dependently reduces HIV-1 infection, indicating that virus-induced ATP release is required for HIV-1 to infect the host cell [37]. Pharmacological addition of ATP or of its nonhydrolyzable analog ATP-gamma-S did not increase the efficiency of HIV-1 infection, suggesting that ATP concentration in the proximity of the plasma membrane was not the limiting factor for virus–cell fusion [37]. It was hypothesized in this work that the released ATP plays a pivotal role in HIV-1 infection by stimulating the P2Y2 receptor subtype, as selective depletion of P2Y2 with siRNA reduced Env-mediated virus–host membrane fusion and eventual HIV-1 infection [37].

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Moreover, incubation of human macrophages with HIV-1 gp120 induced a transient increase in the extracellular ATP concentration within a few minutes where the ATP concentration reached 50 nM in the bulk phase (~~when the virus is~~ not in close contact with the host plasma membrane) [38]. This work further showed that expression of purinergic receptors by the host cell was critical for the entry of HIV-1 into host cells, suggesting that ATP release facilitated viral entry through P2 receptor activation [38]. Further, the work reported that the treatment of macrophages with oxidized ATP (oATP), a P2X inhibitor, reduced viral entry, while treatment with the P2X1 antagonist NF279, the P2X7 antagonist A-740003, or the P2Y1 antagonist MRS 2179

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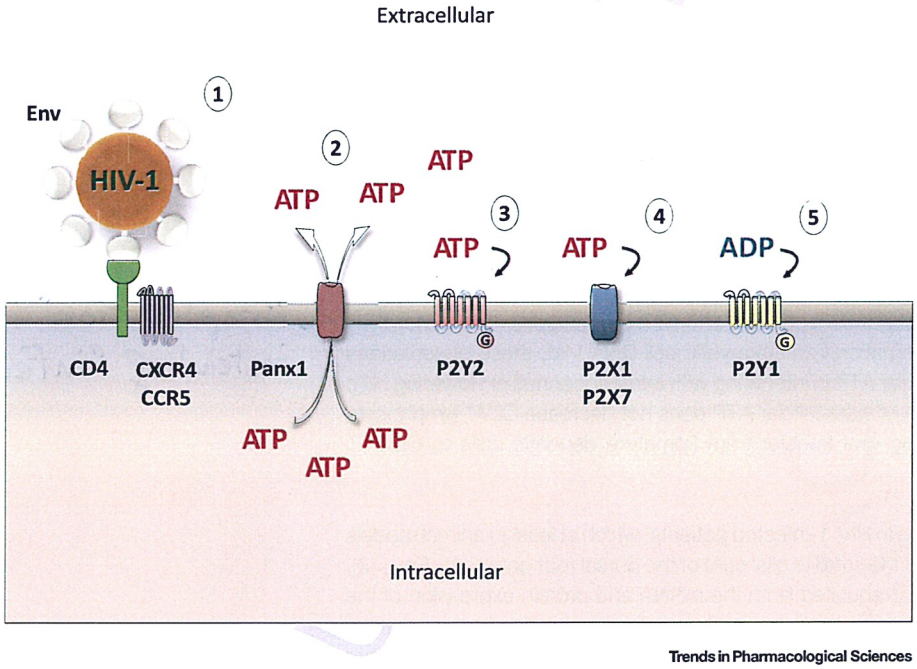

Figure 2. Purinergic Receptors and Nucleotides Involved in HIV-1–Host Cell Interaction. (1) HIV-1 envelope proteins (Env) interact with cell CD4 and chemokine coreceptors (CXCR4 or CCR5). (2) The interaction induces pannexin-1 (Panx-1)-mediated ATP release. (3) ATP in turn signals the P2Y2 receptor and modulates fusion of the virus envelope with the host cell plasma membrane. (4) Activation of P2X1 and P2X7 by ATP and (5) of the P2Y1 subtype by ADP during HIV-1 infection also occurs, which modulates later phases of HIV-1 infection and replication.

significantly reduced HIV-1 replication [38]. These observations initially led to the hypothesis that P2X1 is involved in HIV-1 entry while P2X7 and P2Y1 are required for viral replication [38]. However, at least for NF279, inhibition of HIV-1 infection could not just be due to P2X1 receptor antagonization but rather due to inhibition of binding of the viral particle to CXCR4 or CCR5 [39]. This was suggested by the lack of detectable P2X1 protein expression in cells used in fusion experiments and by the fact that addition or depletion of extracellular ATP did not affect the outcome of viral fusion [39]. The involvement of P2X receptor subtypes in HIV-1 infection of lymphocytes both through cell-free and cell-to-cell contact was also suggested in other work [40]. Virus entry was prevented in a dose-dependent manner when $CD4^+$ T lymphocytes were pretreated with the generic P2X inhibitor pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid tetrasodium salt (PPADS) [41]. PPADS was shown to block HIV-1 infection by impairing virus and host membrane fusion [40].

Additional actors, such as pannexins (Panx) and ectonucleotidases, have been identified and added to the mechanistic model of HIV-1–cell interaction [41–43]. Pannexins are large transmembrane channels that allow the passage of molecules such as ATP across the cell membrane [44,45]. HIV-1 infection of PBMCs and $CD4^+$ lymphocytes causes biphasic opening of pannexin-1 (Panx1) hemichannels [42]. Interestingly, Panx1 was found in virological synapses present between HIV-1-infected and uninfected cells [37]. ATP and its derivatives can permeate through pannexins and then stimulate purinergic receptors [44,45]. Inhibiting it by 4,4-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) or 4-acetamido-4′-isothiocyanatostilbene-2,2′ disulfonic acid (SITS) has been shown to protect cells against HIV-1-induced cell death [37]. Further, while the participation of Panx1 in the release of HIV-1 particles has also been postulated, the mechanism remains to be clarified [37]. Moreover, CD39 activity is found on the surface of HIV-1 particles where its kinetic properties are similar to those of CD39 expressed by macrophages [43]. It has also been shown that inhibition of the CD39 activity of HIV-1 infected macrophages *in vitro* by three ecto-ATPase inhibitors, POM-1, ARL67156, and BG0, decreased HIV-1 infection [43]. Further, the activity of ectonucleosidase NTPDase in HIV-1 was pronounced towards ATP, ADP, and UTP [43].

The addition of ATP to human macrophages induces a decrease in HIV-1 infection [43]. Pretreatment of macrophages with 500 μM ATP for 3 h makes them less prone to HIV-1 ~~infection~~, an effect that is even more pronounced at 24 and 48 h post-infection [43]. Since ATP did not interfere with viral DNA integration into eukaryotic cell DNA [46], these observations suggest an involvement of extracellular ATP in interfering with intraendosomal pH lowering [47]. However, in macrophages, addition of extracellular ATP does not decrease $CD4^+$ lymphocyte infection by HIV-1, instead reducing viral transfer from immature dendritic cells to $CD4^+$ T lymphocytes [47].

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Chronic neuropathic pain is common in HIV-1-infected patients, which at least in animal models can be reproduced by stimulation of the satellite glial cells of the dorsal root ganglia by Env [48]. Incubation of Env with these cells upregulated both the mRNA and protein expression of the P2Y12 receptor and increased mechanical and thermal hyperalgesia, pointing to a role for this receptor in Env-induced modification of pain perception in rats [49]. It is interesting to note that P1 receptors often show antagonistic responses to P2 receptors, such as in the case of decreasing the production of proinflammatory cytokines [50]. It was recently shown that activation of the A_{2A} P1 receptor inhibits tumor necrosis factor alpha (TNF-α) production induced by the HIV-1 Tat protein (known to enhance viral transcription) in a monocytic cell line [51].

Cytomegalovirus (CMV)-Mediated Activation of P2 Network Helps in its Evasion of Immune System

Little is known about the strategies employed by CMV to evade the host immune response and it is speculated that they might at least in part depend on P2 receptors. *In vitro* infection of endothelial cells with CMV upregulated P2Y1, P2Y2, and P2X7 receptors [52]. In addition, enhanced ecto-ATPase and ecto-5′-nucleotidase enzymatic activity has also been observed in CMV-infected endothelial cells [53]. The authors in this work speculated that increased ADO production as a consequence of CMV infection might have antithrombotic and immunosuppressive functions thus helping the virus to evade the host immune system. However, a formal demonstration remains lacking for this hypothesis.

Virus-Mediated Activation of P1 Network Helps in Replication of HSV

The role of P1 purinergic receptors in HSV–host interaction has been highlighted in a rat model [54] and in a study on HSV-1 propagation in various cell lines [55]. HSV-1 orally administered to rodents infects their enteric nervous system and affects gut contractility. Interestingly, ADO-mediated contraction was found to be impaired at 1 and 6 weeks after virus administration and the infection induced a clear redistribution of P1 receptors in the rat enteric nervous system. Expression of both the A_1 and A_{2B} receptors were limited to muscles, whereas A_{2A} and A_3 were mainly present in the myenteric plexus [54]. The infection also increased ADA and CD73 levels in the longitudinal muscle–myenteric plexus [54]. Further ideas about the involvement of P1 receptors in HSV-1 biology came from the observation that the Toll-like receptor 7 (TLR7) agonist imiquimod blocked HSV-1 replication and diffusion by upregulating the antiviral protein cystatin A via an A_1 receptor-mediated pathway [55].

Viral Modulation of the Purinergic Network towards Antiviral Responses

The immune response against microbes can be amplified by ATP [56,57]. Macrophages, the immune cells responsible for recognizing viruses, are not easily activated towards an immune response, suggesting that this activation requires additional signals that can derive from other cells. It has been demonstrated that in the course of adenoviral vector infection in a cocultivated culture of macrophages and epithelial cells, crosstalk between these cells results in potentiation of the inflammatory response due to stimulation of the P2X7 receptor by extracellular ATP [58]. ATP/P2X7 tandemly further stimulate activation of the inflammasome (the protein complex responsible for inflammatory responses) and the secretion of IL-1β. Further support towards the importance of purinergic signaling in host immune modulation came from a study in mice where, following intranasal administration of replication-deficient adenoviral vectors, exacerbation of the immune response caused acute respiratory distress syndrome with a consequent high mortality in wild-type but not in P2X7-knockout (KO) mice. Furthermore, a decrease in important markers of inflammation, such as neutrophil infiltration and IL-1β and IL-6 levels, was observed [59]. However, it is to be noted that the amplification of the immune response against viruses by P2 receptors [56,57] can have both beneficial and detrimental effects on tissues depending on the level of activation of the immune system. Overactivation of the host innate immune response to viral infection can have devastating consequences on tissues [57]; however, mild and controlled activation has been shown to improve the antiviral responses of the cells. We review examples of both cases below.

Excessive Amplification of Antiviral Immune Response

Asthma is a disease characterized by overactivation of the immune system that leads to inflammation of the airways. Participation of P2X7 receptors in asthma has been nicely demonstrated [60,61]. Additional work also shows that P2X7 is important in asthma control during naturally occurring viral infection of the upper respiratory tract [62]. Asthmatic subjects with loss-of-function

or attenuated P2X7 receptor activity exhibited a higher risk of loss of asthma control, while a better prognosis was predicted for patients with normal P2X7 function [62].

Controlled Amplification of Antiviral Immune Responses

Activation of P2X7 by ATP and P2Y6 receptors by UDP has been shown to limit the replication of vesicular stomatitis virus (VSV) both *in vitro* and *in vivo* [63,64]. Moreover, pharmacological addition of ATP (in the micromolar range) before virus infection has been shown to decrease the replication of VSV, Newcastle disease virus (NCV), murine leukemia virus (MLV), and HSV in a P2X7-dependent manner. Furthermore, in P2X7-KO mice the replication of VSV was not reduced [63]. These observations were attributed to the production of the antiviral cytokine interferon beta (IFN-β), which was increased in a concentration- and time-dependent manner in cell lines stimulated with ATP [63]. Cells infected with VSV also released UDP in the micromolar range by a Panx1-mediated mechanism that upregulated the P2Y6 receptor [64]. Pretreatment of cells with micromolar concentrations of UDP protected cells from VSV infection, while knockdown of P2Y6 promoted VSV infection and replication [64]. These observations led to the hypothesis that UDP promotes the secretion of IFN-β thus enhancing antiviral immunity against VSV [64].

Acute encephalitis caused by rabies virus (RV) in mammals has a high fatality rate. As repeated vaccinations are needed to induce a sufficient antibody response against the virus, it is crucial to find new systems to enhance antibody concentration in the blood. Experimental vaccination in mice has shown that the serum antibody concentration can be significantly increased by injecting inactivated rabies vaccine with UTP, but not with ATP, and that this effect was inhibited by anti-P2Y4 receptor antibodies, suggesting that UTP acts as an adjuvant by enhancing the Th2-mediated humoral immune response in mice [65].

Dengue fever is a tropical mosquito-borne viral infectious disease [66]. The host immune system responds against an infection by dengue virus (DV) by secreting IFN-γ. Work has shown that inhibition of the P2X7 receptor significantly inhibited the anti-DV IFN-γ response [67]. Furthermore, *in vitro* prestimulation of human monocytes with extracellular ATP induced a significant decrease in viral load that was more pronounced in P2X7-expressing cells, pointing to their involvement in this effect [68].

Human respiratory syncytial virus (RSV) is the most important etiological agent of bronchiolitis and childhood pneumonia [69]. RSV infection of primary human bronchial epithelial cultures induces the development of goblet cells with enhanced secretion of mucin, release of ATP, and accumulation of ADO [70]. RSV infection of the bronchoalveolar epithelium causes reduced basal alveolar fluid clearance with an increase in mucus fluid, air congestion, and rhinorrhea. In mice, inhibition of *de novo* pyrimidine synthesis by leflunomide prevented UTP release and virus-induced basal alveolar fluid clearance, suggesting that the reduction in RSV-induced alveolar fluid clearance is mediated by the extracellular UTP activating P2Y receptors expressed by the bronchoalveolar cells [71]. Accordingly, antagonization of these receptors by suramin or XAMR-0721 (a competitive antagonist of P2Y receptors) or by hydrolysis of UTP prevented RSV-induced inhibition of basal alveolar fluid clearance. This effect was reverted by addition of UTP (in the nanomolar range), and peculiarly the effect was not mimicked by ATP, suggesting that it was fully dependent on UTP-activated P2Y receptors [72].

Concluding Remarks and Future Perspectives

Infection by viruses induce both short- and long-term ATP release from the infected cells [63,64]. This release is mediated mostly by vesicular exocytosis and by the activation of hemichannel Panx1 [63,73]. Besides ATP, the presence of its metabolites ADP, AMP, and

ADO in the supernatants of HIV-1 as well as of other viruses shows that ectonucleotidases remain active during infection [74]. In some cases, virus infection is accompanied by upregulation of ectonucleotidases resulting in ATP degradation and formation of its byproducts, particularly ADO, whose concentration increase is likely to provide the virus with a favorable environment (i.e., anti-inflammatory and antiaggregatory conditions) ~~inside the infected cell~~.

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Published research has consistently shown that both P2X and P2Y (particularly the P2Y2 subtype [37]) receptors are critically involved in virus entry and replication. The HIV-1–human cell interaction has been extensively investigated to shed light on the role of P2 receptors in cell entry and replication in human macrophages and $CD4^+$ T lymphocytes [37,3,74,75]. To enter the host cell and move into the cytoplasm, HIV-1 exploits the endocytic activities of the host cell. Therefore, it might be possible that autocrine low or pulsed activation of the purinergic receptors of the infected cell might promote actin reorganization by activating Ca^{2+}-dependent intracellular cascades thus facilitating membrane ruffling and viral particle endocytosis. Here, low, localized, and pulsed ATP release could be needed at precise time points for viral entry and replication (Figure 3A). According to this model, ATP-releasing molecules and the P2 purinergic receptors could be considered as facilitators of viral infection. Therefore, interruption of ATP release and the subsequent autocrine purinergic receptor activation could give new opportunities to counteract viral penetration into the cell [37,41]. Another important finding is that *in vitro* treatment of cells with purinergic receptor antagonists and blockers (e.g., oATP, suramin, PPADS) greatly reduces HIV-1 infection and replicative capacity without affecting the viability of the infected cells [37,40]. Therefore, *in vivo* and clinical studies investigating the response of these compounds towards different viruses is a strong therapeutic avenue towards the prevention and treatment of viral diseases (see Outstanding Questions).

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Patients surviving HIV-1 infection undergo premature aging and show augmented risk for pathologies dependent on or related to chronic inflammation such as heart disease, bone disease, and cancer [40,76]. Since purinergic signaling has been indicated as a crucial trigger for acute and chronic inflammation [13,22], the use of P2 inhibitors may have the double positive effect of decreasing viral infection and reducing the eventual deleterious inflammatory responses. Towards this purpose, it is critical to study the link between P2 receptors involved in inflammatory responses and the exacerbation of virus-induced disease symptoms [62]. Further, it is known that about half of HIV-1-infected patients undergo impairment in cognitive function, ranging from mild neurocognitive disorders to HIV-associated dementia and other central nervous system diseases [77]. The pathophysiology underlying these changes could be due at least in part to neurotoxic mediators such as ATP and glutamate, both of which are released from HIV-1-infected macrophages and/or by stressed/dying neurons. It might be possible to use P2 receptor blockers to reduce purinergic-mediated glutamate release and its deleterious effects on neurons [74]. It also known that ADO and P1 receptors have anti-inflammatory roles and mediate tissue protection against immune-mediated damage, particularly in the brain [78,79]. This has been demonstrated in a viral model of multiple sclerosis, opening the possibility that P1 receptors can also be investigated as therapeutic targets in future for other neurological diseases [28].

An important and apparently contrasting observation is that pharmacological treatment of host cells with ATP before viral contact reduces viral infection efficiency and the production of new virions (Figure 3B). Pharmacological administration of ATP causes elevated membrane stress and/or cell death, impairs viral loading, and stimulates chemotaxis of macrophages, neutrophils, and microglia, hence activating the cell immune system and enhancing the production of antiviral cytokines [35,63,64,68]. Therefore, the clear message emerging from the field is that

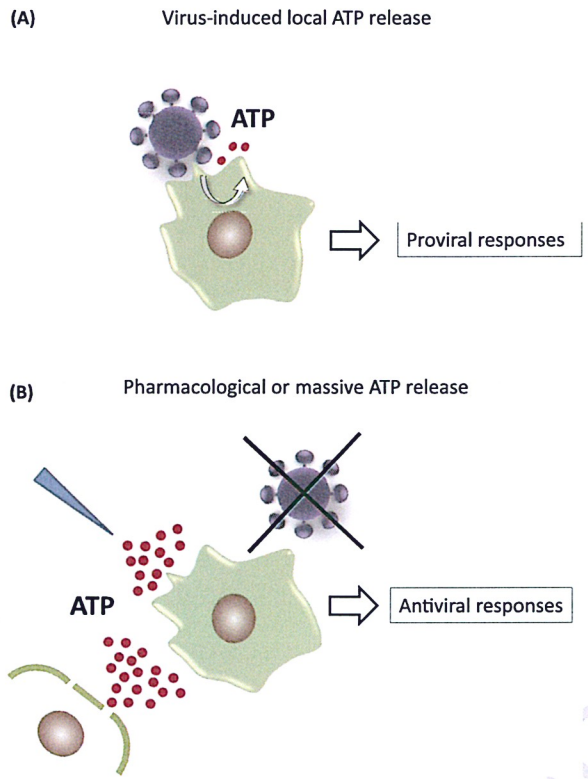

Figure 3. Model of Action of Extracellular ATP in Viruses. (A) Local and regulated release of low ATP concentrations induced by interaction of the virus with the host cell favors viral penetration and replication, while (B) pharmacological stimulation of P2-expressing cells with ATP or release of high ATP concentrations induced by extensive cell damage induces defensive responses (transcription of antiviral cytokine genes, production of reactive mediators, chemotaxis), thus reducing the efficiency of viral infection and replication.

Outstanding Questions

Is it possible to pharmacologically target specific or multiple purinergic receptors to block cell infection by viruses?

Does inhibition of purinergic receptors improve the pathological consequences of viral infection?

Would inactivation of extracellular ADO modulate viral function?

Does ATP impair the cell-killing capacity of virus-infected cells by NK and $CD8^+$ T lymphocytes?

Would nucleotide employment improve large-scale vaccine production?

the purinergic network of the eukaryotic cell represents a potential new pharmacological target to control viral infections.

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Acknowledgments

The authors apologize to the researchers whose excellent work could not be cited owing to space limitation. D.F. is supported by local funds from the University of Ferrara. P.M. is supported by the Italian Ministry of Education University and Research (PRIN2015).

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