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Role of Connexin/Pannexin containing channels in infectious diseases

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Abstract

In recent years it has become evident that gap junctions and hemichannels, in concert with extracellular ATP and purinergic receptors, play key roles in several physiological processes and pathological conditions. However, only recently has their importance in infectious diseases been explored, likely because early reports indicated that connexin containing channels were completely inactivated under inflammatory conditions, and therefore no further research was performed. However, recent evidence indicates that several infectious agents take advantage of these communication systems to enhance inflammation and apoptosis, as well as to participate in the infectious cycle of several pathogens. In the current review, we will discuss the role of these channels/receptors in the pathogenesis of several infectious diseases and the possibilities of generating novel therapeutic approaches to reduce or prevent these diseases.

Keywords

Virus; bacteria; purinergic; gap junctions; HIV; CMV; AIDS; reservoirs; pathogen

General introduction

Gap junction (GJ) channels are formed by the docking of two hemichannels, each contributed by one cell. Hemichannels consist of hexamers of homologous subunit proteins, termed connexins (Cxs), that connect the cytoplasm of adjacent cells [1, 2]. In addition, it was shown that unapposed hemichannels (uHC), before their cell-to-cell docking to form GJ, are also open on cell surface, allowing exchange of small factors between the cytoplasm and the extracellular environment. In addition to the connexin family, pannexin also can

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form uHCs. Both types of channels, GJ and uHC (connexin and pannexin containing), have an internal pore of approximately 12 Å, allowing ions and intracellular messengers up to ~1 kDa in molecular mass to diffuse between connected cells or from the cytoplasm into the extracellular space [1, 2]. The diffusion of these second messengers results in the coordination of multiple physiological functions [2].

ATP is a second messenger exchanged by GJ and uHC and is the main purinergic messenger. ATP is released into the extracellular space as a neurotransmitter, but also in conditions of apoptosis and inflammation by exocytosis, transporters, and opening of uHC. Once released into the extracellular space, ATP binds to purinergic receptors and is degraded by ectonucleotidases to produce ADP, AMP, and adenosine. These molecules activate specific purinergic and adenosine receptors. Purinergic receptors are divided into metabotropic (adenosine and purinergic P₂Y) and ionotropic receptors (purinergic P₂X). Adenosine receptors, also named P₁ receptors, are seven transmembrane metabotropic receptors coupled to G_i, G_s and G_o proteins. P₂ receptors are divided into P₂X and P₂Y receptors and are activated by ATP, ADP, and UTP. P₂X receptors are ATP-gated cationic channels and are associated with uHC that upon opening provide the source of intracellular ATP for purinergic receptor activation. P₂Y receptors are activated by ATP, ADP, UTP, and UDP, and several subtypes of these receptors work together with uHC providing/releasing ATP as a ligand.

Here, we will review the mechanism by which GJ and uHC in concert with purinergic receptors and extracellular ATP (or subproduct of its degradation) participate in several infectious diseases and on the potential of these channels to be used as therapeutic targets to reduce or prevent infectious diseases.

Inflammation and down regulation of Connexin containing channels:

Historic perspective

Several publications in the 1990's demonstrated that LPS and inflammation decreased gap junctional communication (GJC) and Cx expression in liver and heart [3–8]. In addition, experiments in brain associated damage such as ischemia/reperfusion, cancer and inflammatory diseases, also indicated that inflammation/damage reduced GJC and Cx expression [9, 10]. Recent reports examining several pathogens including *Bordetella pertussis*, *Helicobacter pylori*, and several viruses also supported the idea that infection with several pathogens resulted in the total shutdown of Cx mediated communication [11]. However, at the same time several manuscripts described unusual results of increased expression of Cx43 in inflammatory conditions in liver, kidney and lungs [12–15], suggesting that not all Cxs were affected equally by inflammation/damage. Later studies indicated that Cxs are expressed in leukocytes [13], microglia [16–19], polymorphonuclear cells [20], monocyte/macrophages [21], T cells [22], and Kupffer cells [14, 15] under inflammatory conditions to coordinate inflammation and immune response. These observations suggest that parenchymal Cxs are negatively affected by inflammation/damage, but immune cells that normally do not express Cxs are prone to express and form functional channels in inflammatory conditions. Only recently, it has been reported that particular pathogens such as *Shigella*, Human immunodeficiency virus (HIV), and *Pseudomonas*

aeruginosa use or increased this communication system to enhance associated inflammation or to participate in the infectious cycle (see review by [11]). Thus, a better understanding of the role of these channels and their association with purinergic receptors and uHC in physiological and pathological conditions it was required.

Purinergic receptors and hemichannels: working together in physiological and pathological conditions

Purinergic receptors participate in several physiological events such as vasodilation, pain, neuroprotection, cell differentiation, migration, muscle contraction, T cell differentiation, platelet aggregation, inflammation, autocrine and paracrine signaling, neurotransmission, apoptosis and several immune responses [23]. Due to their importance in physiological processes, alterations in expression and function of these receptors result in pathological conditions, including epilepsy, excessive pain, hypertension, and several infectious diseases.

Response to infectious agents or inflammation can be summarized in several steps including initial damage or infection, release of chemoattractants, recruitment of leukocytes, transmigration and subsequent local inflammation/clearance of the agent or damaged area. Normally in the blood, erythrocytes circulate in the periphery to exchange nutrients and oxygen/CO₂. In inflammatory conditions, hemodynamics change and circulating white cells interact with the endothelial surface in a process called margination. After this change in localization, leukocytes become adherent and start to transmigrate into areas of damage or infection by traversing the endothelial cells and basement membrane. During all these processes the presence and participation of Cx and Panx containing channels, extracellular ATP, and purinergic receptors has been described as discussed below.

The role of extracellular ATP, uHC, and purinergic receptors in inflammation and infectious disease has only recently been examined and recognized. Purinergic receptors and uHC are highly expressed in immune cells and play a key role in migration as well as killing of intracellular pathogens. The best examined purinergic receptor is P₂X₇ due to its early identification and relatively easy evaluation of gating due its similar pore size as compared to uHC. P₂X₇ is widely expressed in hematopoietic and nervous systems and participates in physiological and pathological processes in concert with uHC. Opening of both P₂X₇ and uHC normally is low to undetectable mainly due to high concentrations of calcium, magnesium and the lack of inflammatory signals (see [11] for an excellent review about regulation of these channels). However, upon opening of uHC, intracellular signaling molecules such as ATP, NADH, PGE₂, and ions are released into the extracellular space, resulting in activation of several surface receptors, including purinergic receptors.

Several infectious agents have evolved to block or overactivate these communication systems by releasing several enzymes that modulate extracellular concentrations of ATP, such as adenylate kinase, ATPase, and 5'-nucleotidase [24–27], altering the communication between uHC and purinergic receptors. Some of these pathogens are *Mycobacterium tuberculosis*, *Shigella flexneri*, *Yersinia enterocolitica*, *Staphylococcus aureus* and several viruses (see details below). Another mechanism of pathogen escape is by direct regulation of the expression and function of purinergic receptors as described for cytomegalovirus [28].

Lastly, several pathogens can regulate ATP secretion as described for *Plasmodium falciparum* or *P. berghei* [29, 30]. It has been proposed that the mechanism by which the anti-malarial drug, mefloquine, works is by inhibiting uHC and purinergic receptors [31], altering infectivity and disease progression, suggesting a key role of these channels in the pathogenesis of malaria. In addition, several pathogens such as HIV, Cytomegalovirus (CMV), *P. aeruginosa*, *S. aureus* and *Escherichia coli*, as well as *Streptococcus pneumoniae*, *Clostridium*, *Yersinia enterocolitica* and *Shigella flexneri* use connexin and pannexin containing channels to spread infection, inflammation and toxicity (see details in [11]). Thus, several potential mechanisms involving the interaction of uHC, ATP and purinergic receptors are “hijacked” by pathogens to survive, replicate and enhance inflammation (see Figure 1).

Cytokine production and inflammation

The best described mechanisms of participation of purinergic receptors and uHC in disease progression are described for cellular migration of immune cells, detection of apoptotic cells, and secretion of inflammatory factors. For example, processing and release of IL-1 β and IL-18 is mediated by secretion of ATP, activation of P₂X₇, opening of uHC and elevated release of intracellular ATP [32–36]. In contrast, chronic release of ATP or inflammation activates cells of the monocyte/macrophage lineage to secrete antiinflammatory cytokines including IL-10 and IL-1 β receptor antagonist to reduce inflammation and causes development of a Th₂ response.

As indicated above, a key element in inflammation is the synthesis, processing and release of IL-1 β . This cytokine is produced as two isoforms, pro-IL-1 α and pro-IL-1 β , which are subsequently cleaved by interleukin-converting enzyme (ICE), also known as caspase-1, to produce the mature isoforms. However, some pathogens like rhinoviruses evolved to induce IL-1 β secretion without a P₂X₇ component [37], suggesting that some viruses can skip the activation of purinergic receptors to trigger inflammation. In addition to this cytokine, IL-18, also known as interferon- γ inducing factor, is also formed by the processing of pro-IL-18 by ICE. As discussed later on, a P₂X₇ polymorphism produces significantly less IL-18 when stimulated by ATP, suggesting a key role in immune response and inflammation. This individual to individual genetic variability, especially in P₂X₇ receptors, is essential to understanding differential responses of particular populations to several pathogens. In addition, polymorphic changes in uHC have been described and may contribute to this variability in immune responses. For example, Cx37 C1019T polymorphism contributes to *Helicobacter pylori* infection in patients with gastric cancer [38]. These observations suggest that genetic alterations in these channels and receptors could alter inflammation and susceptibility to several infectious agents.

Inflammasome activation requires ATP-purinergic receptors to trigger shedding of microvesicles [34], TLR-4 activation [39], and apoptosis [40]. Also, ATP impairs IL-10 expression of soluble and membrane bound HLA-G Ag in human monocytes [41], suggesting that ATP or pathogens altering ATP secretion/stability could alter immune responses mediated by the immunosuppressive activity of IL-10. ATP also inhibits IL-12 and IL-23 expression in human dendritic cells by a cAMP dependent mechanism [42],

suggesting that ATP also plays a key role in immunosuppression. In addition, IL-10 suppresses IL-2 secretion [43] by interfering with CD28 induced activation [44]. Thus, by targeting uHC, extracellular ATP, and purinergic receptors some pathogens may alter NFAT T cell activation, resulting in anergy, senescence and apoptosis [45, 46].

Another critical event in inflammation and infection is leukocyte migration into areas of damage. Most leukocytes migrate in response to chemoattractants including chemokines, lipids (e.g. lysophosphatidylcholine released by apoptotic cells) and nucleotides (e.g. ATP and UTP) [47, 48]. Several lines of evidence indicate that uHC and purinergic receptors participate in these events. Migration of human monocytes in response to CCL2/MCP-1 across the blood brain barrier is dependent on Cx43 containing gap junction channels, but we cannot discard the participation of uHC [21]. Further, Cx43 containing channels and purinergic receptors modulate neutrophil recruitment/activation. Subsets of CD4⁺ T cells express Cx43 and form functional GJ with macrophages [22, 49, 50]. MCP-1/CCL2 synthesis and release in rat alveolar and peritoneal macrophages is P₂Y₂ dependent [51]. Thus, leukocyte migration is regulated by purinergic receptors, ATP, and uHC during all stages of inflammation. Our data indicate that several chemokines including RANTES, MIPs, and SDF-1 cause transient opening of pannexin-1 uHC in human leukocytes [52]. We propose that this transient uHC opening is associated with cellular activation and migration, but further studies are required to test this hypothesis.

In early apoptosis, release of nucleotides occurs by opening of pannexin-1 uHC. During later events of apoptosis this channel is targeted by caspase-3 and -7, proteases that cleave an intracellular portion of the channel, contributing further to the apoptotic process by disrupting the gating of the channel [48, 53]. Thus, during the apoptotic process uHC, ATP, and purinergic receptors participate as regulators of chemoattraction as well as key regulators of recognition and clearance of apoptotic cells. In addition, there are several reports that describe the potential of purinergic receptors and/or uHC to interact with intracellular adaptor proteins, such as laminin, actin, actinin, integrins, kinases and phosphatases [54, 55], to amplify inflammation and damage but the significance of these interactions is still unclear. All these areas are under active investigation.

Tuberculosis

Mycobacterium tuberculosis is the infectious agent that causes tuberculosis in humans. In this infectious disease, activation of P₂X₇ receptors play a key role in inducing intracellular killing of this bacterium inside of macrophages by a calcium dependent mechanism that induces phagosome-lysosome fusion [56–58]. This response to mycobacterial infection is dependent on polymorphic changes in the P₂X₇ receptor [59]. In addition, several splicing variants of P₂X₇ have been described as having different agonist dependency and interaction with pannexin-1 uHC [60], suggesting that these changes may also contribute to the pathogenesis of tuberculosis. Pannexin-1 uHC also has several genetic isoforms [61], suggesting that the interaction of this complex between purinergic and uHC is dependent on the genetic background of each individual, providing additional risk and protective host factors against tuberculosis. Further, the allele 1513C of P₂X₇ is a risk factor in the development of extrapulmonary and pulmonary tuberculosis in several ethnic populations,

including north Indian, Mexican, Russian, and Turkish [62]. In contrast, P₂X₇ polymorphism in position -762 is protective against tuberculosis in an Asian-Indian cohort [63].

Similar effects of extracellular ATP and purinergic receptors have been described in clearance and immune response to *Chlamydia* by a phospho-lipase D dependent mechanism, resulting in 70–90% elimination of this intracellular bacterium in macrophages [64–66]. However, several pathogens can “hijack” these systems. For example, *Chlamydia* infected macrophages are protected from apoptosis while uninfected cells are still susceptible to cell death [65]. Similar survival mechanisms were observed in HIV infected cells to generate reservoirs to perpetuate replication and pathogen survival [67–69].

Shigella and Yersinia

These are gram negative bacteria that are transmitted mainly by the fecaloral route, resulting in diarrhea and fever by a mechanism involving invasion and replication in the colon with epithelial dysfunction. Infection of epithelial cells with *S. flexneri* results in Cx43, 32 and 26 uHC activity that supports invasion, by actin and PLC dependent mechanisms [70], resulting in ATP release that helps bacterial invasion and replication [70, 71]. In addition, infected cells induce a bystander activation of uninfected cells resulting in secretion of IL-18 by a gap junction dependent mechanism [72]. It is critical to mention that secretion of IL-18 is also dependent on purinergic receptor activation as described above. Interestingly, the mechanism of *Shigella flexneri* uptake was dependent on micropodial extensions from the cell surface (like filopodiums) allowing complexes of proteins in the tips of these processes to bind the bacteria by an ATP and Cx dependent mechanism. This local release of ATP activates Erk1/2 and results in actin retrograde flow of these processes that are in contact with the bacteria. In cases of infection with *Yersinia enterocolitica*, opening of Cx43 uHC has been described by a mechanism dependent on channel phosphorylation [73]. Thus, both entero-pathogens utilize the complex interactions of uHC, ATP, and purinergic receptors, as well as GJ channels to invade, replicate and amplify dysfunction in the host.

Staphylococcus aureus

This gram positive bacterium lives in every healthy human. Due to the wide use of antibiotics, methicillin resistant *S. aureus* (MRSA) has emerged. Mainly found in hospitals and several sensitive communities, a key pathogenic component of this bacterium is the peptidoglycan present in the bacterial cell wall. Peptidoglycan reduces Cx expression (protein and mRNA) and functional gap junction coupling in astrocytes by a mechanism involving p38 MAPK [74]. Studies in brain slices also demonstrated down regulation of gap junctional communication in response to peptidoglycan [75]. However, despite the reduction in GJC, increased expression of Cx43 and Cx30 as well as opening of uHC has been reported [75, 76]. In contrast, peptidoglycan induces expression of Cxs that result in functional gap junction communication in microglia, suggesting that depending of the cell type and type of the insult, these channels could participate in both physiological and pathological conditions.

Cytomegalovirus (CMV)

This usually harmless virus is a double-stranded DNA virus and member of the herpes virus family. Around 50–80% of the population is infected. Viral transmission occurs by contact with saliva, urine, breast milk and other fluids. 0.2 to 1.2% of all births are CMV contaminated and no treatment is available. However, congenital CMV infection is a major public health problem that results in permanent problems such as hearing loss, and mental/developmental disabilities. It is clear that hearing loss is attributable to congenital CMV infection and is associated with heterozygote Cx26 mutations as compared to congenital CMV infected children without hearing loss [77], suggesting that potential genetic changes induced by CMV infection may alter Cx26 channels, including uHC and GJ. Further, dysfunction of Cx26 uHC, GJ, and purinergic receptors has been associated with mild to severe hearing/deafness [78, 79], suggesting a potential link between both conditions.

CMV infection is a strong promoter of degradation of Cx43 and disruption of GJC [80]. Central and peripheral nervous system development is highly dependent in Cx43 GJ and perhaps uHC [81, 82], and we propose that CMV infection may compromise this development [83]. We propose that CMV alters Cx43 trafficking and function, resulting in CNS compromise often observed in CMV infected children. This is a new area of research that requires priority due to the significant contribution of the virus to nervous system and hearing problems.

Other viral and bacterial infections

As indicated above both viral and bacterial infection can reduce communication between uHC, GJ, purinergic receptors, and alter secretion of ATP. Swine Influenza viral infection down-regulates endothelial Cx43 expression by increased degradation in a ERK dependent manner [84]. Borna virus also down regulates Cx36 in specific CNS cell types [85–87]. Influenza during pregnancy alters development of the brain of the fetus, suggesting that viruses can impact neuronal development by affecting GJs [88] possibly by a similar mechanism as proposed for CMV. Studies in Vero cells demonstrated that infection with Herpes Simplex Virus-2 (HSV-2) and treatment with the antiviral agent, acyclovir, down-regulated GJ channels and Cxs expression [89–91]. It was reported that an increase in tyrosine phosphorylation by HSV-2 and Rous sarcoma virus leads to an inhibition of GJ and Cx43 expression [92–94]. Bovine papillomavirus type 4 E8, when bound to ductin, causes loss of GJ between primary fibroblasts [95, 96]. As indicated above most viruses reduced communication and expression of these channels/receptors contributing to inflammation and parenchymal dysfunction.

HIV pathogenesis

HIV is a retrovirus that causes acquired immunodeficiency syndrome (AIDS), a condition that mainly attacks the immune system allowing opportunistic infections, cancer and aging related diseases to rise due to lack of an effective immune system. Normally, HIV infects a variety of immune and non-immune cells, including CD4⁺ T cells, macrophages, microglia, and astrocytes.

Our results in HIV CNS infection indicate that Cx containing channels, gap junctions and uHC play a key role in amplification of inflammation and apoptosis. In the CNS, microglial cell and perivascular macrophages are the predominant cell types infected by HIV [97, 98]. However, a small population of HIV infected astrocytes has been detected [98–106]. The importance of these few infected astrocytes in the pathogenesis of NeuroAIDS has not been examined extensively. Our data indicates that astrocytes are minimally infected by HIV (5–8%), but despite this low infection, HIV uses GJ to spread toxicity and inflammation into uninfected surrounding cells, such as astrocytes and endothelial cells of the blood brain barrier [107]. The small number of HIV infected astrocytes resulted in large alterations in glutamate metabolism and CCL2 secretion, supporting the idea that HIV infection of astrocytes is essential for neuronal compromise and leukocyte transmigration, respectively. Interestingly, HIV infected cells are protected from apoptosis by an unknown mechanism [69, 108, 109]. We propose that these surviving cells generate CNS viral reservoirs within the CNS. We recently identified the intracellular signals that mediate these toxic effects as Cytochrome C dependent molecules, including IP₃ and intracellular calcium [67].

In addition, we showed that HIV infection of astrocytes results in the opening of Cx43 uHC, secretion of ATP, and inflammation. Opening of Cx43 uHC requires interaction of the virus with an unknown receptor(s) on the membrane of astrocytes. Microarray analysis indicated that dickkopf-1 protein (DKK1, a soluble wnt pathway inhibitor) mediates compromise of neuronal processes in neurons mediated by HIV infection of astrocytes [110]. Previous to our work, DKK1 also was identified as a key regulator of IFN- γ enhancement of HIV replication and reactivation in astrocytes [111], suggesting that DKK1 also plays a role in HIV replication in astrocytes. The findings that Cxs participate in the pathogenesis of NeuroAIDS open a new avenue of investigation to study the mechanisms by which HIV “hijacks” this communication system, GJ and uHC, to spread toxicity, inflammation, and to increase leukocyte transmigration into the CNS, and may suggest novel therapeutic approaches to target NeuroAIDS.

Our data and others indicates that HIV binding to CD4⁺ T lymphocytes and macrophages also results in activation of pannexin-1 uHC, release of ATP, and activation of specific purinergic receptors [112–114]. Exposure of CD4⁺ T lymphocytes to HIV results in the biphasic opening of pannexin-1 uHC: early (5–15 min) during the interaction of the virus with CD4 and CCR5 or CXCR4, depending of the viral strain used and late, during the release of new virions into the medium (18 to 24 h). Blocking pannexin-1 uHC totally abolished viral replication. Experiments in human macrophages also indicate that HIV infection requires secretion of ATP and activation of purinergic receptors. We demonstrated that P₂X₁ receptors are essential for HIV entry into macrophages. However, we did not identify where in the viral cell cycle, P₂X₇ and P₂Y₁ are regulating replication. In addition, activation of P₂Y₂ has been also associated with HIV infection and replication [112].

In contrast, activation of adenosine receptors is protective against HIV tat induced toxicity of neurons [115, 116], suggesting that adenosine may also play an important role in preventing HIV-tat neurotoxicity within the CNS. In addition, activation of adenosine receptors down regulates surface expression of CXCR4 and CCR5 on CD4⁺ T lymphocytes [117], suggesting that adenosine is protective against HIV infection. Thus, further studies

are required to design new therapeutic approaches to reduce and block HIV infection and replication in immune and CNS cells.

Conclusions and future

The findings that uHC containing pannexins or connexins working in concert with purinergic receptors and extracellular ATP (see Figure 1) still is a poorly explored area and requires further investigation. In addition, it is not surprising that these components are misused by several pathogens that have evolved to take advantage of this system to coordinate infection, replication, and associated inflammation. Several pharmaceutical companies already developed drugs to target several purinergic receptors as well as uHC (probenecid) for different diseases, including vascular dysfunctions and arthritis related diseases. However, we still require specific agonists and antagonists as well as the identification of the mechanisms by which pathogens alter these pathways, in order to design, in an informed manner, novel therapeutic approaches to reduce the devastating consequences of infectious diseases. In contrast, several compounds that blocks purinergic receptors are consumed daily without control as food dyes, including compounds derivate from brilliant blue G and Brilliant Blue FCF that are present in several foods and drinks. The consequences of the ingestion of these blockers is unknown but we can predict that reducing immune activation and elimination of cancer cells, could results in depressed immune response and faster cancer evolution amount several potential diseases affected.

The relatively recent discovery of these complexes between purinergic receptors, extracellular ATP and hemichannels, opens new avenues to understand not only inflammation, but several diseases including pathologies with impaired cellular migration, inflammation, and cell clearance (cancer, regeneration and aging). The implications that several pathogens also “highjacks” these complexes indicates that targeting these proteins also could prevent and reduce infectious processes. Thus, the possibility of targeting these complexes of proteins to prevent and treat infectious diseases emerges as a novel therapeutic form to treat infectious agents.

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Figure 1.

Proposed mechanism by which pathogens use extracellular ATP, uHC and purinergic receptor in the context of infectious diseases. Extracellular and intracellular pathogens bind or activate signaling related to these receptors, leading to ATP release through uHC containing connexin or pannexin proteins. Extracellular ATP binds to specific purinergic receptors, activating calcium influx and G coupled proteins that facilitate intracellular signaling. This signaling participates in inflammation and several pathogens life cycles. In addition, upon release of ATP, several extracellular enzymes degrade ATP into ADP, AMP, and adenosine, which further activate other purinergic and adenosine receptors that participate in inflammation and pathogenesis.