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Review

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.

1. 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 unopposed 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 form uHCs. Both types of channels, GJ and uHC (connexin and pannexin containing), have an internal pore of approximately 12 A°, 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 P_2Y) and ionotropic receptors (purinergic P_2X). Adenosine receptors, also named P1 receptors, are seven transmembrane metabotropic receptors coupled to G_i, G_s and G_o proteins. P_2 receptors are divided into P_2X and P_2Y receptors and are activated by ATP, ADP, and UTP. P_2X receptors are ATP – gated cationic channels and are associated with uHC that upon opening provide the source of intracellular ATP for purinergic receptor activation. P_2Y 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.

2. Inflammation and down regulation of Connexin containing channels: historic perspective

Several publications in the 1990s 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

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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.

3. 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/CO_2. 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 P_2X_7 due to its early identification and relatively easy evaluation of gating due its similar pore size as compared to uHC. P_2X_7 is widely expressed in hematopoietic and nervous systems and participates in physiological and pathological processes in concert with uHC. Opening of both P_2X_7 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, PGE_2, 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 *Plasmodium 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), *Plasmodium auruginosa*, *S. aureus* and *Escherichia coli*, as well as *Streptococcus pneumonia*, *Clostridium*, *Y. enterocolitica* and *S. 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 Fig. 1).

3.1. 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-1β and IL-18 is mediated by secretion of ATP, activation of P_2X_7, 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 anti-inflammatory cytokines including IL-10 and IL-1β receptor antagonist to reduce inflammation and causes development of a Th_2 response.

As indicated above, a key element in inflammation is the synthesis, processing and release of IL-1β. This cytokine is produced as two isoforms, pro-IL-1α and pro-IL-1β, which are subsequently

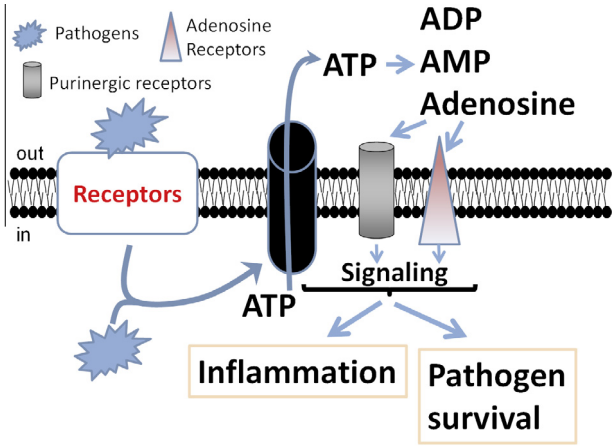

Fig. 1. Proposed mechanism by which pathogens use extracellular ATP, uHC and purinergic receptor in the context of infectious diseases. Extracellular and intracellular pathogens bind or activate signaling related to these receptors, leading to ATP release through uHC containing connexin or pannexin proteins. 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.

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