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Extracellular ATP and adenosine: The Yin and Yang in immune responses?

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ABSTRACT

Extracellular adenosine 5′-triphosphate (ATP) and adenosine molecules are intimately involved in immune responses. ATP is mostly a pro-inflammatory molecule and is released during hypoxic condition and by necrotic cells, as well as by activated immune cells and endothelial cells. However, under certain conditions, for instance at low concentrations or at prolonged exposure, ATP may also have anti-inflammatory properties. Extracellular ATP can activate both P2X and P2Y purinergic receptors. Extracellular ATP can be hydrolyzed into adenosine in a two-step enzymatic process involving the ectonucleotidases CD39 (ecto-apyrase) and CD73. These enzymes are expressed by many cell types, including endothelial cells and immune cells.

The counterpart of ATP is adenosine, which is produced by breakdown of intra- or extracellular ATP. Adenosine has mainly anti-inflammatory effects by binding to the adenosine, or P1, receptors (A1, A2A, A2B, and A3). These receptors are also expressed in many cells, including immune cells. The final effect of ATP and adenosine in immune responses depends on the fine regulatory balance between the 2 molecules. In the present review, we will discuss the current knowledge on the role of these 2 molecules in the immune responses.

1. Introduction

Extracellular adenosine and ATP exert important functions in physiology and pathophysiology. They are for instance important in heart and vascular function (Erlinge and Burnstock, 2008), during pregnancy (Spaans et al., 2014a) and in immune responses (Junger, 2011). Under physiological conditions, very low concentrations of extracellular ATP (400–1000 nM) (Bakker et al., 2007; Ryan et al., 1996) and adenosine (40–80 nM) (Ontyd and Schrader, 1984) are found. Upon for instance tissue stress, *e.g.* necrosis or apoptosis (Gallucci and Matzinger, 2001), hypoxia or inflammation (Bours et al., 2006), ATP is released from cells into the extracellular environment leading to 3 or more fold increase in extracellular ATP concentrations (Bodin and Burnstock, 1998). This is for instance seen in inflammatory diseases, such as atherosclerosis (Jalkanen et al., 2015) and preeclampsia (Bakker et al., 2007). Extracellularly, ATP acts as a Danger Associated Molecular Patterns (DAMPs) (Bours et al., 2006; Jacob et al., 2013) and can bind to purinergic receptors (P2 receptors) and initiate signaling cascades to induce an inflammatory response (Jacob et al., 2013). To avoid ATP-induced pathological effects, ATP can be hydrolyzed into adenosine and phosphate by a cascade of enzymes (Yegutkin, 2008), which are expressed in many tissues. Thus the final effects of ATP and adenosine on the immune response depend on the balance between ATP and adenosine in the immediate vicinity of the cells. The present review will discuss the current knowledge on the roles of ATP and adenosine in immune responses.

2. Adenosine and ATP balance

ATP is actively released from activated or stressed cells, i.e.

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during inflammation, hypoxia or apoptosis and passively from necrotic cells, via ruptured cell membranes (Gallucci and Matzinger, 2001; Lazarowski, 2012) (Fig. 1). Active release from activated or apoptotic cells is done via mainly two mechanisms: exocytosis of intracellular vesicles or transport via membrane bound channels or transporters (Lazarowski, 2012). These mechanisms may act together in the same cell, since Ledderose et al. recently showed that stimulated ATP release from immune cells occurred in 2 phases: instantaneous ATP release followed by a later, second phase of ATP release, suggesting two different mechanism of ATP release (Ledderose et al., 2015). Exocytotic release of ATP into the extracellular environment has been shown for many cell types, such as neuronal cells (Gualix et al., 1999), platelets (Dean et al., 1984), lymphocytes (Tokunaga et al., 2010), mast cells (Anderson et al., 1974) and endothelial cells (Bodin and Burnstock, 2001). These cells store ATP in extracellular vesicles, which is released into the extracellular environment upon vesicular exocytosis, in which the vesicles are incorporated into the plasma membrane and the content is released into the extracellular environment.

Conductive release of ATP from cells is associated with 2 types of plasma membrane channels, the Cl^- channels, such as maxi-ion channels and volume-regulated ion channels, or the pore forming channels, such as connexins and pannexins. The maxi-ion channels have been found in many cell types, such as endothelial cells (Bahamonde and Valverde, 2003), placental cells (Riquelme, 2009), and in various immune cells (McCann et al., 1989; Kolb and Ubl, 1987; Schlichter et al., 1990). These channels allow passage of small organic anions, such as ATP (Sabirov and Okada, 2004), and are activated by e.g. osmotic swelling (Islam et al., 2012) and during hypoxia (Dutta et al., 2004). Volume regulated channels are also permeable to organic anions, including ATP, and are also activated by osmotic swelling (Hisadome et al., 2002). They have been shown to be present in endothelial cells (Hisadome et al., 2002) and macrophages (Burow et al., 2015).

Connexins are known to be the building blocks of gap-junctions, however, some connexin channels appear to be localized to non-junctional regions of the plasma membrane to form channels, which are involved in shuttling of molecules to the extracellular environment (Herve, 2004). These channels may typically respond to membrane depolarization (Contreras et al., 2003) or lowering of the extracellular Ca^{2+} concentration (Muller et al., 2002). ATP release from these channels can also be induced by certain pro-inflammatory stimuli (Robertson et al., 2010). Certain types of connexin channels, such as Cx43 or Cx32, have been shown to be involved in the extracellular release of ATP (Kang et al., 2008; De Vuyst et al., 2006). Connexin channels are for instance expressed by vascular smooth muscle and endothelial cells (Haefliger et al., 2004). ATP release via these channels has been shown to play a role in pathological conditions, such as atherosclerosis (Wong et al., 2006). Also pannexin units form channels, which are permeable to ATP (Bao et al., 2004). Pannexin channels have been found in many cell types, including immune cells (Chen et al., 2015; Woehrle et al., 2010). ATP release from pannexin channels has been shown to be induced by various stimuli, such as hypoxia (Sridharan et al., 2010) or apoptosis (Chekeni et al., 2010; Sandilos et al., 2012).

Once extracellularly, ATP can be dephosphorylated into ADP, AMP and adenosine by different enzymes. ATP can be dephosphorylated into ADP and AMP by ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1 or CD39) and alkaline phosphatase (AP) (Yegutkin, 2008). AMP can be further dephosphorylated to adenosine by 5'-ectonucleotidase (or CD73) (Yegutkin, 2008) (Fig. 2). The bioavailability of extracellular adenosine is regulated by either degradation of adenosine by adenosine deaminase (ADA) into inosine or transport into the cells by nucleoside transporters (Hirschhorn and Ratech, 1980; Latini and Pedata, 2001) (Fig. 2).

3. Adenosine and ATP receptors

In the extracellular environment, adenosine binds to P1 receptors, a family of G protein-coupled receptors (Table 1). This family consists of 4 subtypes, A1, A2A, A2B and A3 (Ralevic and Burnstock, 1998). A1 and A2A are high affinity receptors, while A2B and A3 are low affinity receptors (Ralevic and Burnstock, 1998). These receptors act via adenylyl cyclase (AC) activity and thus modulate cyclic AMP. A2a and A2b stimulate AC, while A1 and A3 inhibit AC (Ralevic and Burnstock, 1998). Thus depending on the presence of receptors on a specific cell, cyclic AMP production can be modulated. P1 receptors are present on a variety of immune cells (Table 1). Functional physiological roles of the P1 receptors have been studied in knock-out mice. The A1, A2 and A3 receptor knock-out mice are all viable, showing no gross abnormalities and are fertile (Day et al., 2003; Johansson et al., 2001; Salvatore et al., 2000); the A1 receptor knock-out mice, however, seem to show accelerated ageing (Gimenez-Llort et al., 2002).

In the extracellular compartment, ATP binds to P2 purinergic receptors (Table 2). There are 2 subsets of P2 receptors: P2X or P2Y receptors (Idzko et al., 2014). P2X receptors are plasma membrane channels that are activated solely by ATP to mediate the influx or efflux of various cations (Na^+, Ca^{2+}, K^+) in cells (Surprenant and North, 2009). In humans and rodents, seven P2X receptor subunits exist (P2X1–P2X7), which form homomeric or heteromeric receptors (Surprenant and North, 2009). P2X receptors are arranged as trimers, with three receptors located around an ion permeable channel. Due to binding of 3 ATP molecules, the

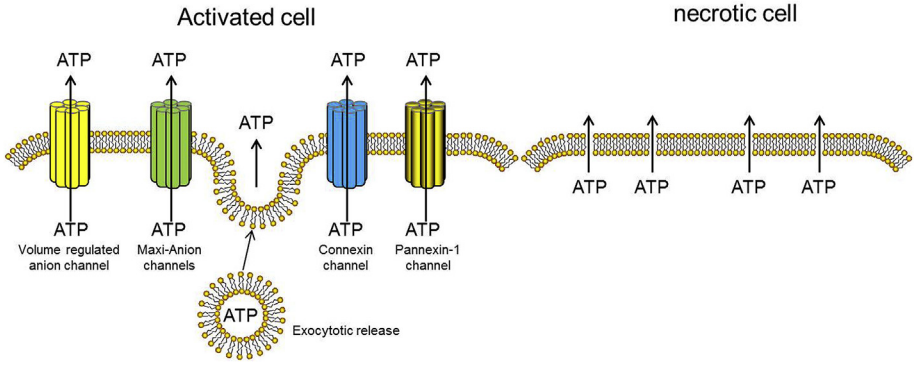

Fig. 1. ATP release from cells. ATP can be released from activated and necrotic cells. The release of ATP from necrotic cells is a passive process, while the release of ATP from activated cells is an active process, which is carried out by volume regulated anion channels, maxi-anion channels, connexin channels or pannexin channels. Moreover, exocytotic release of ATP has also been observed in cells.