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Lipopolysaccharide alters nucleotidase activities from lymphocytes and serum of rats

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Abstract

ATP exerts a proinflammatory role and induces cytokine release by acting at $P2X_7$ receptors. The product of ATP hydrolysis is the nucleoside adenosine, an important immunomodulator. The main source of extracellular adenosine is the hydrolysis of extracellular ATP by a group of ecto-enzymes: ENTPDase family, NPP family and ecto-5′-nucleotidase. Considering the role of ATP and adenosine in inflammatory processes, we investigated the effect of lipopolysaccharide on ectonucleotidases activities and expression in lymphocytes from mesenteric lymph nodes and serum of rats, in order to better understand the involvement of extracellular nucleotide hydrolysis in an endotoxemia model. We observed significant changes on nucleotidase activities from lymphocytes and serum of rats after in vitro and in vivo exposure to LPS. In vitro results have shown an increase on nucleotide hydrolysis in lymphocytes and a decrease on the enzyme activity of NPP in blood serum. In vivo, we observed an increase on nucleotide hydrolysis in lymphocytes and a decrease in the hydrolysis of all nucleotides tested in blood serum. After 24 and 48 h of LPS treatment, there was a reduction in NTPDase1, 2, 3 and ecto-5′-nucleotidase transcripts. These results suggest that there is a time-dependent enhancement of extracellular nucleotides metabolism in lymphocytes and blood serum after the induction of an endotoxemic model. The changes observed suggest that these enzymes can act in the regulation of extracellular nucleosides and nucleotides in a model able to trigger inflammatory process.

Keywords: LPS; ATP; Adenosine; NTPDase; NPP; 5′-nucleotidase; Endotoxemia

Introduction

Extracellular ribonucleotides, such as ATP and UTP, have been considered as a new class of signaling molecules that might play a role in inflammation. Ribonucleotides are released at sites of inflammation as a result of cell damage (Di Virgilio et al., 2001). Virtually all cell types express surface receptors for these signaling ribonucleotides. Among these nucleotide receptors, the group of P2 nucleotide receptors comprises P2Y G-protein coupled receptors (P2YR) and the P2X receptors (P2XR), which are ligand-gated ion channels (Ralevic and Burnstock, 1998). P2 receptors are involved in cytokine release (Ferrari et al., 2000; Solle et al., 2001), chemotaxis (McCloskey et al., 1999), vasodilation (Marrelli, 2001; Buvinic et al., 2002), apoptosis (Molloy et al., 1994; von Albertini et al., 1998), T-cell activation and proliferation (Baricordi et al., 1999; Harada et al., 2000), and dendritic cell function (Ferrari et al., 2000). There is convincing evidence that ATP exerts a proinflammatory role and induces cytokine release by acting at $P2X_7$ receptors (Solle et al., 2001).

The product of ATP hydrolysis is the nucleoside adenosine, an important signaling molecule. Adenosine acts through four G-protein-coupled adenosine receptors (A_1, A_{2A}, A_{2B} and A_3) (Fredholm et al., 2001). Adenosine receptors mediate their

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signals by heteromeric G-proteins that can either stimulate (Gs) or inhibit (Gi) adenylyl cyclase. Activation of adenosine receptors on myeloid and lymphoid cells has also been shown to modulate inflammation (Haskó and Cronstein, 2004; Sitkovsky et al., 2004). Endogenous adenosine exerts a significant proportion of its anti-inflammatory actions via binding to A_{2A} receptors found on vascular endothelium, epithelium, monocytes/macrophages, neutrophils, mast cells, lymphocytes, platelets and neurons (Sullivan, 2003). Furthermore, the actions of adenosine receptors are viewed as protecting cardiovascular and neuronal tissues from hypoxia or injury, which can increase the local concentration of extracellular adenosine by 10–100-fold (Fredholm et al., 2001).

The main source of extracellular adenosine is the hydrolysis of extracellular ATP by a group of ecto-enzymes: the ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) family, the ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP) family and the ecto-5′-nucleotidase (EC 3.1.3.5) (Zimmermann, 2001). NTPDases have an important role in cell adhesion and in controlling lymphocytes function, including antigen recognition and/or the effectors activation of cytotoxic T cells (Dombrowski et al., 1995). Furthermore, NTPDases play an important role in lymphocytes, since extracellular nucleotides are mediators of immune and non-immune cell function (Dombrowski et al., 1998). Four members of the family are tightly bound to the plasma membrane via two transmembrane domains, and have a large extracellular region with an active site facing the extracellular milieu. NTPDase1, 3 and 8 slightly prefer ATP over ADP by a ratio of 1, 3 and 2, respectively. Meanwhile, NTPDase2 prefers triphosphonucleosides (Bigonnesse et al., 2004; Vorhoff et al., 2005). NTPDase 1 (CD39) was originally identified as an activation marker for B-lymphocytes and subsequently shown to be expressed on subsets of activated NK (natural killer) cells, T-lymphocytes, epidermal Langerhans DC (dendritic cells) and endothelial cells (Maliszewski et al., 1994). CD39 has also been shown to modulate vascular inflammation, cellular proliferation and migration (Goepfert et al., 2000, 2001) and to play a crucial role in the regulation of the ADP-purinoreceptor P2Y1 function (Enjyoji et al., 1999; Schulte am Esch et al., 1999).

E-NPPs have multiple physiological roles, including nucleotide recycling, modulation of purinergic receptor signaling, regulation of extracellular pyrophosphate levels, stimulation of cell motility, and possible roles in regulation of insulin receptor signaling and activity of ecto-kinases (Goding et al., 2003). Ecto-5′-nucleotidase, otherwise known as CD73, is a lymphocyte maturation marker, which is involved in intracellular signaling, lymphocyte proliferation and activation (Airas, 1998; Resta et al., 1998).

Considering the role of ATP and adenosine in inflammatory processes, we investigated the effect of lipopolysaccharide endotoxin from *Escherichia coli* on ectonucleotidases in lymphocytes and serum from adult rats. Furthermore, we evaluate the E-NTPDases and ecto-5′-nucleotidase expression in mesenteric lymph nodes, in order to better understand the involvement of extracellular nucleotide hydrolysis in an endotoxemia model.

Materials and methods

Animals

In all experiments, male Wistar rats of approximately 60–70 days old, weighing around 250 g from our breeding stock were used and housed four to a cage, with water and food ad libitum. The animal house was kept on a 12 h light/dark cycle (lights on at 7:00 am) at a temperature of 23 ± 1 °C. Procedures for the care and use of animals were adopted according to the regulations of Colégio Brasileiro de Experimentação Animal (COBEA), based on the Guide for the Care and Use of Laboratory Animals (National Research Council).

In vitro experiments

Different concentrations of LPS (from *E. coli*, serotype 0111:B4) (25, 50, 75 and 100 μg/ml) were tested on nucleotidase activities in serum and lymphocytes from mesenteric lymph nodes of naïve rats. LPS was preincubated with reaction medium during 10 min and, immediately after, the enzyme assays were performed.

In vivo experiments

Rats were injected intraperitoneally with either LPS (2 mg/kg body weight) (Spolarics et al., 1996) or saline. The animals were killed 24 and 48 h after injection.

Isolation of blood serum fraction

Blood samples were drawn after decapitation of rats and were soon centrifuged in plastic tubes at 5000 *g* for 5 min at 20 °C. The serum samples obtained were then stored on ice and immediately used in the experiments (Oses et al., 2004).

Isolation of lymphocytes

Mesenteric lymph nodes were removed and passed through a mesh grid in saline 0.9% (Wu et al., 1991). Cells were washed three times with saline, centrifuged at 200 *g* for 10 min. After, cells were centrifuged two times at 200 *g* for 10 min with the same buffer used in the enzyme assays, without divalent cations. The cells were counted with Trypan Blue and only the groups with more of 95% of viability were used for the experiments.

Enzyme assays

Measurement of serum ρ-Nph-5′-TMP hydrolysis

ρ-Nph-5′-TMP hydrolysis was determined essentially as described by Sakura et al. (1998). The reaction mixture containing ρ-Nph-5′-TMP, as a substrate (at the final concentration of 0.5 mM) in 100 mM Tris–HCl, pH 8.9, was incubated with approximately 1.0 mg of serum protein at 37 °C for 5 min in a final volume at 200 μl. The reaction was stopped by the addition of 200 μl of NaOH 0.2 N. The amount