Immunopharmacology and Inflammation

Adenosine A<sub>2A</sub> receptor agonist (CGS-21680) prevents endotoxin-induced effects on nucleotidase activities in mouse lymphocytes

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A R T I C L E   I N F O

Article history:
Received 18 February 2010
Received in revised form 20 October 2010
Accepted 3 November 2010
Available online 27 November 2010

Keywords:
Lipopolysaccharide
Lymphocytes
Nucleoside triphosphate diphosphohydrolase
Nucleotide pyrophosphatase/phosphodiesterase
5′-Nucleotidase
Adenosine

A B S T R A C T

Adenosine 5′-triphosphate (ATP) released during inflammation presents proinflammatory properties. Adenosine, produced by catabolism of ATP, is an anti-inflammatory compound. Considering the role of ATP and adenosine in inflammation and the importance of ectonucleotidases in the maintenance of their extracellular levels, we investigated the effect of a selective agonist of the adenosine A<sub>2A</sub> receptor (CGS-21680) on ectonucleotidase activities and gene expression patterns in lymphocytes from mice submitted to an endotoxia model. Animals were injected intraperitoneally with 12 mg/kg Lipopolysaccharide (LPS) and/or 0.5 mg/kg CGS-21680 or saline. Nucleotidase activities were determined in lymphocytes from mesenteric lymph nodes and analysis of ectonucleotidase expression was carried out by a semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Exposure to endotoxia promoted an increase in nucleotide hydrolysis. When CGS-21680 was administered concomitantly with LPS, this increase was prevented for ATP, adenosine 5′-monophosphate (AMP), and p-Nitrophenyl thymidine 5′-monophosphate (p-Nph-5′-TMP) hydrolysis. However, when CGS-21680 was administered 24 h after LPS injection, the increase was not reversed. The expression pattern of ectonucleotidases was not altered between LPS and LPS plus CGS-21680 groups, indicating that the transcriptional control was not involved on the effect exerted for CGS-21680. These results showed an enhancement of extracellular nucleotide catabolism in lymphocytes after induction of endotoxia, which was prevented, but not reversed by CGS-21680 administration. These findings suggest that the control of nucleotide and nucleoside levels exerted by CGS-21680 could contribute to the modulation of the inflammatory process promoted by adenosine A<sub>2A</sub> agonists.

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1. Introduction

Inflammation is a defense response devised to protect the integrity of the organism against endogenous or exogenous noxious agents (Medzhitov, 2008). The inflammatory response comprises vascular and cellular reactions finely tuned by several mediators, such as chemical factors derived from plasma proteins or cells. Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is a molecule that triggers innate immunity and plays an adjuvant effect on adaptive immunity (Condie et al., 1968).

The role of purinergic signaling in the regulation of immune and inflammatory responses has become evident. An increase in ATP release during inflammation has been described and this compound presents proinflammatory properties (Bodin and Burnstock, 1998). Adenosine is a nucleoside formed by the enzymatic breakdown of ATP that acts as a neuromodulator in the central and peripheral nervous system. It has been reported that tissue damage and inflammation are accompanied by accumulation of extracellular adenosine due to its release from non-immune and immune cells (Sitkovsky, 2003). Adenosine interacts with four different G-protein-coupled receptors: A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub> (Fredholm et al., 2001). Because this nucleoside presents anti-inflammatory properties, acting mainly on adenosine A<sub>2A</sub> receptors, a role of adenosine in the control of inflammation has been suggested (Sullivan, 2003; Thiel et al., 2003; Capecci et al., 2006). Furthermore, it has been proposed that the administration of adenosine A<sub>2A</sub> agonists could be useful in inflammatory events and sepsis (Thiel et al., 2003; Sullivan et al., 2004).
The presence of nucleotide-metabolizing pathways on the surface of immune and non-immune cells, which also co-express ATP and adenosine receptors, is essential for the fine-tuning regulation of the duration and magnitude of purinergic signaling. Several enzyme families are involved in the control of nucleotide and nucleoside levels: the ecto-nucleoside triphosphate diphosphohydrolases (E-NTDPase), the ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP) and the ecto-5′-nucleotidase (EC 3.1.3.5) (Zimmermann, 2001). E-NTDPases have an important role in cell adhesion and in controlling lymphocyte function, including antigen recognition and/or the effector activation of cytotoxic T cells (Dombrowski et al., 1995; Dombrowski et al., 1998). Four members (E-NTDPase1-3 and 8) 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. 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 the regulation of insulin receptor signaling and the activity of ecto-kinases (Godin et al., 2003). Ecto-5′-nucleotidase (CD73) is a lymphocyte maturation marker, which is involved in intracellular signaling, lymphocyte proliferation and activation (Airas, 1998; Resta et al., 1998).

Considering the roles that the nucleotides and nucleosides play during inflammatory events, and the importance of ectonucleotidas for the maintenance of extracellular levels of the former, we investigated the effect of a selective agonist of the adenosine A2A receptor (CGS-21680) on ectonucleotidase activities in lymphocytes from mice submitted to an endotoxemia model. Furthermore, the effect of LPS and CGS-21680 on the E-NTDPase, E-NPP, and ecto-5′-nucleotidase gene expression were also evaluated in mesenteric lymph nodes.

2. Materials and methods

2.1. Chemicals

CGS-21680 hydrochloride (3′-4′,5′-cyclophosphoryloxy-5′-ethylcarbamoyl)-3,4-dihydroxy-oxlano-2-yl[purin-2-yl]amino[ethyl]phenyl[propanoic acid], HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), Lipopolysaccharide (LPS) from Escherichia coli, serotype 0111:B4, nucleotides (ATP, ADP, and AMP), Malachite Green, Trizma Base, and p-Nitrophosphonic acid 5′-monophosphate (p-Nph-5′-TMP) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The RNAspin Ilustra Mini Kit for RNA isolation was purchased from GE Healthcare. dNTPs, oligonucleotides, Taq polymerase, Low DNA Mass Ladder, and SuperScript™ III First-Strand Synthesis SuperMix were purchased from Invitrogen (Carlsbad, CA, USA). Primers were obtained from Integrated DNA Technologies (Coralville, IA, USA) and GelliRed™ was purchased from Biotium (Hayward, CA, USA). The LDH Liqueuniform Kit was purchased from Labtest Diagnostica S.A. (Lagoa Santa, MG, Brazil). All reagents were of analytical grade.

2.2. Animals

In all experiments, male F1 mice (approximately 8–10 weeks old, weighing around 50 g) from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS, Porto Alegre, RS, Brazil) 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 the Colégio Brasileiro de Experimentação Animal (COBEA), based on the Guide for the Care and Use of Laboratory Animals (National Research Council) and all efforts were made to minimize the number of animals used in this study and their suffering. This study was approved by the Ethics Committee of Universidade Federal do Rio Grande do Sul (UFRGS) under license number 2006628.

2.3. Experimental protocols

The animals received intraperitoneal (i.p.) injections of saline (0.9%), LPS (12 mg/kg) (Pawlinski et al., 2003), and CGS-21680 (0.5 mg/kg body weight) (Martire et al., 2007), according to the groups described below. All solutions were administered in a volume of 2 ml/kg body weight. Mice were randomly divided in: (i) control group (SAL), which received a single saline injection, (ii) CGS group (CGS), which received a single injection of CGS-21680, (iii) LPS group (LPS 24 h), which was submitted to the endotoxemia model by a single injection of LPS, and (iv) LPS + CGS (LPS + CGS 24 h), which was submitted to the endotoxemia model by a single injection of LPS and 24 h later received a single injection of saline, and (vi) LPS + CGS (LPS + CGS 48 h), which was submitted to the endotoxemia model by a single injection of LPS and 24 h later received a single injection of CGS-21680. All animals were euthanized by decapitation 24 h after the last injection.

2.4. Isolation of lymphocytes

Mesenteric lymph nodes were removed and passed through a mesh grid in wash buffer (the same buffer used in the enzyme assays, without divalent cations). Cells were washed two times with this buffer by centrifugation at 200 g for 10 min. After, the cells were stained with 0.1% Trypan Blue and counted, and only the groups with more than 95% viability were used in the experiments.

2.5. Assays of ecto-nucleoside triphosphate diphosphohydrolase (E-NTDPase) and ecto-5′-nucleotidase activities

The reaction medium contained 1 mM CaCl₂ (for ATP and ADP hydrolysis) or MgCl₂ (for AMP hydrolysis), 120 mM NaCl, 5 mM KCl, 60 mM glucose, 1 mM sodium azide, 0.1% mM albumin, and 20 mM Hepes buffer, pH 7.5, in a final volume of 200 μl. Approximately 10⁶ lymphocytes were added to the reaction medium and the enzyme reaction was started by the addition of ATP, ADP or AMP to a final concentration of 2 mM, followed by incubation for 30 min at 37 °C. The reaction was stopped with 200 μl of 10% trichloroacetic acid (TCA). Incubation times, protein concentrations, reaction mixtures, and the phosphodiesterase activity were assessed using a colorimetric method as previously outlined by Chan et al. (1986). Controls to correct for non-enzymatic substrate hydrolysis were performed by adding the cells after the reactions had been stopped with TCA. All reactions were performed in triplicate. Enzyme activities were generally expressed as nmol Pi released per min per 10⁵ cells.

2.6. Assay of ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP) activity

The phosphodiesterase activity was assessed using p-Nph-5′-TMP (an artificial substrate). The reaction medium contained 1 mM CaCl₂, 120 mM NaCl, 5 mM KCl, 60 mM glucose, 1 mM sodium azide, 0.1% mM albumin, and 20 mM Tris buffer, pH 8.9, in a final volume of 200 μl. Approximately 10⁶ lymphocytes were added to the reaction medium and the enzyme reaction was started by the addition of p-Nph-5′-TMP to a final concentration of 0.5 mM. After 60 min of incubation, 200 μl of 0.2 N NaOH were added to the medium to stop the reaction. Incubation time and protein concentration were chosen in order to ensure the linearity of the reaction. The amount of p-nitrophenol released from the substrate was measured at 400 nm
using a molar extinction coefficient of 18.8×10⁻⁳/M/cm. Controls to correct for non-enzymatic substrate hydrolysis were performed by adding the cells after the reaction had been stopped with NaOH. All reactions were performed in triplicate. Enzyme activity was generally expressed as nmol β-nitrophenol released per min per 10⁶ cells (Sakura et al., 1998; Vuaden et al., 2009).

2.7. Analysis of gene expression by semi-quantitative RT-PCR

Analysis of NTPDase1 (Entpd1), 2 (Entpd2), 3 (Entpd3), 8 (Entpd8), NPP1 (Enpp1), 2 (Enpp2), 3 (Enpp3), and 5-nucleotidase (Nt5e) gene expression was carried out by a semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Twenty-four and/or 48 h after treatments, mesenteric lymph nodes of mice (n=3 for each group) were removed for total RNA extraction with the RNAspin Ilustra Mini Kit in accordance with the manufacturer’s instructions. RNA purity was quantified spectrophotometrically and assessed by electrophoresis in a 1.0% agarose gel using GelRed™. The cDNA species were synthesized using SuperScript™ III First-Strand Synthesis SuperMix from 3 μg of total RNA following the supplier’s instructions. For PCR assays, 1 μl of cDNA was used as a template and screened with specific primers for Entpd1, 2, 3, 8, Enpp1, 2, 3 and Nt5e. PCR reactions were carried out in a volume of 25 μl using a concentration of 0.2 μM of each primer, 200 μM MgCl₂, and 1 U Taq polymerase. The cycling conditions for all PCRs were as follows: Initial 1 min denaturation step at 94 °C, 1 min at 94 °C, 1 min annealing step (Entpd1, 2, Enpp1 and Actb: 63 °C; Entpd3, Enpp3 and Nt5e: 62 °C; Entpd8: 64 °C; Enpp: 61 °C), 1 min extension step at 72 °C. These steps were repeated for 35 cycles. Finally, a 10 min post-extension step was performed at 72 °C. Primer sequences as well as the amplification products are listed in Table 1. Ten microliters of the PCR reaction mixture were analyzed on a 1% agarose gel using GelRed™ and photographed under UV light. The Low DNA Mass Ladder was used as a molecular marker and normalization was performed employing Actb (β-actin) as a constitutive gene. The images of stained PCR products were analyzed by optical densitometry and semi-quantified (enzyme/Actb mRNA ratios) using the computer software Image J.

2.8. Statistical analysis

Results are expressed as means±standard error (S.E.M.). Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by a Tukey multiple range test. Statistically significant differences between groups were considered for a P<0.05.

Table 1

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sequence (5′-3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entpd1</td>
<td>Sense GGT GGC TGC CTT AAG GAC CCG TGC</td>
</tr>
<tr>
<td>Entpd1</td>
<td>Antisense GGA GCT TGC TGT AAG ITT ATA GCC TGG TCG</td>
</tr>
<tr>
<td>Enpp1</td>
<td>Sense CCA CTG TCA GCC TGT CAG CAG CCA GC</td>
</tr>
<tr>
<td>Enpp2</td>
<td>Antisense ACC GCC TTC ACC TGG GGC CAT C</td>
</tr>
<tr>
<td>Enpp3</td>
<td>Antisense GCT GAC AAG CAG TAG GAC CGG CCA TAC</td>
</tr>
<tr>
<td>Enpp1</td>
<td>Sense TAT TGG CTA TGG ACC TCG TCT CTT CAA GA</td>
</tr>
<tr>
<td>Enpp1</td>
<td>Antisense GTG TAA TCC GGG GCC TCC CTT AG</td>
</tr>
<tr>
<td>Enpp2</td>
<td>Sense CTC TCT TAG TCG TCA ACC CAG C</td>
</tr>
<tr>
<td>Enpp2</td>
<td>Antisense CGT CGG TGC TAG AGA CCT CAG CTT G</td>
</tr>
<tr>
<td>Enpp3</td>
<td>Sense ACA TGC AGG AGA GGT GTC AAC CCC TGC</td>
</tr>
<tr>
<td>Enpp3</td>
<td>Antisense AGA AGA GTG TAT CAA CTC CAT AGC GTC</td>
</tr>
<tr>
<td>Nt5e</td>
<td>Sense CCA TCA GCG GCC AGA ACC TGG CTC</td>
</tr>
<tr>
<td>Nt5e</td>
<td>Antisense CTT CAT CGG CCC TCC TTC AAC GGC TG</td>
</tr>
<tr>
<td>Actb</td>
<td>Sense GTG CCA TGT TCG TCT AGA CTT CCA CCA GG</td>
</tr>
<tr>
<td>Actb</td>
<td>Antisense CAC CGA TCA ACA CAG ACT TGC CAG C</td>
</tr>
</tbody>
</table>

3. Results

3.1. Cellular integrity

The lymphocyte preparation integrity was checked by measuring lymphocyte lactate dehydrogenase (LDH) activity. The ratio of this enzyme activity measured in intact and disrupted lymphocytes can be regarded as a measure of damaged particles. The protocol was carried out according to the manufacturer’s instructions. Triton X-100 (1%, final concentration) was used to disrupt the lymphocyte preparation. The measurement of LDH activity showed that most cells (approximately 90%, n=3) were intact after the isolation procedure (data not shown).

3.2. Effect of LPS-induced endotoxemia model and CGS-21680 on ectonucleotidase activities in lymphocytes

After 24 h of LPS exposure we observed a significant increase in ATP hydrolysis (178%) when compared with the control group. This increase was prevented when CGS-21680 was co-administered with LPS. Likewise, after 48 h of LPS exposure, we observed a significant increase in ATP hydrolysis (135%) when compared with control, although this was not reversed by the administration of CGS-21680 (Fig. 1A). Fig. 1B shows that when compared to control ADP hydrolysis increased significantly after induction of endotoxia with LPS. However, there was no significant difference between groups that received LPS plus CGS-21680 and the groups that received LPS only.

![Fig. 1](image-url)
3.3. Effect of LPS and CGS-21680 on ectonucleotidase mRNA expression in mesenteric lymph nodes

The results show that mRNA transcript levels were altered in after LPS and CGS-21680 treatments when compared to saline group for Entpd3 and Enpp3 (Fig. 4A and B). For others enzyme mRNA transcripts we did not observe significant alterations in mesenteric lymph nodes after the treatments (data not shown). We examined the Entpd8 transcripts in liver (as a positive control) and in mesenteric lymph nodes. However, we found that Entpd8 was not expressed in lymph nodes (data not shown).

4. Discussion

In the present study, we observed that LPS-induced endotoxemia model increased the nucleotide hydrolysis promoted by E-NTPDases, E-NPP and ecto-5′-nucleotidase activities in lymphocytes of mice. In addition, the co-administration of CGS-21680 with LPS was able to prevent the increase of enzyme activities.

A role for the purinergic system in the immune response has become more accepted over the last few years. In a brief update, Di Virgilio et al. (2007) reported that interest in this hypothesis is slowly growing among the immunological community, as observed by the increase in the number of papers reporting the effect of purinergic agonists on many different immune-mediated responses. It has been demonstrated that extracellular ATP is accumulated at sites of inflammation and induces an inflammatory response, being, in relevant amounts, considered a signal of tissue injury or distress (Di Virgilio et al., 2009). Other work has shown that ATP acts as an immunomodulatory agent via P2X and P2Y receptors, more specifically via the P2X7 subtype (Ferrari et al., 2006). In addition, adenosine, the final product of ATP hydrolysis, exerts anti-inflammatory effects via A2A receptors (Di Virgilio et al., 2009).
The enzymes responsible for ATP hydrolysis are named ectonucleotidases (NTPDase family, NPP family and ecto-5′-nucleotidase) (Zimmermann, 2001). Besides their involvement in the role of ATP in inflammation, enzymes that degrade extracellular nucleotides, such as NTPDase1 (CD39) and 5′-nucleotidase (CD73), present immunomodulatory activity (Dwyer et al., 2007). Furthermore, NTPDases play an important role in lymphocyte function, since extracellular nucleotides are mediators of immune and non-immune cell function (Dombrowski et al., 1998).

Our results demonstrated a significant increase in nucleotide hydrolysis in lymphocytes after mice had been exposed to endotoxemia model, both at 24 h and 48 h after LPS exposure. A similar result was observed previously in our laboratory, when rats were injected with LPS and the hydrolysis of ATP, ADP, and AMP in lymphocytes from mesenteric lymph nodes was determined later (Vuaden et al., 2007). This increase in nucleotide hydrolysis could be related to a compensatory response, decreasing the availability of ATP, a proinflammatory agent, and, consequently, contributing to the production of extracellular adenosine, an anti-inflammatory compound. Here we investigated the effect of CGS-21680, a selective A2A adenosine receptor agonist, on nucleotide catabolism. When CGS-21680 was administered alone, we did not observe a significant difference compared to the control group. However, when CGS-21680 was administered concomitantly with LPS, it was able to prevent the LPS-induced increase in ATP, AMP, and p-Nph-5′-TMP hydrolysis (LPS + CGS 24 h group). In contrast, when CGS-21680 was administered 24 h after LPS injection, the increase was not reversed (LPS + CGS 48 h group). Therefore, we suggest that this selective A2A agonist can prevent the endotoxic effects of LPS, but cannot reverse such effects when the endotoxemia model has already been established. Despite the effects observed on ATP hydrolysis, there was no significant difference between LPS and LPS plus CGS-21680 groups for ADP hydrolysis. Since it has been reported that E-NTPDases present different abilities to hydrolyze tri and diphosphonucleosides (Zimmermann, 2001), we cannot exclude the possibility that LPS might up-regulate the ectonucleotidase activities and that the co-administration of CGS-21680 could prevent the LPS-induced effect over distinct E-NTPDase members.

In order to evaluate whether the endotoxemia model and CGS-21680 could alter the ectonucleotidase gene expression, we performed RT-PCR assays. The gene expression pattern of ectonucleotidases presented an increase in mRNA levels in groups treated with LPS and LPS plus CGS-21680 mainly for Entpd3 and Enpp3. For other enzyme mRNA transcript levels analyzed, the differences between treated groups and control group were not so evident. Interestingly, these results differ from the rat model of endotoxemia in which we previously demonstrated that LPS decreased the Entpd and Nt5e mRNA levels from rat lymph nodes (Vuaden et al., 2007). Although LPS also induced an increase on ectonucleotidase activities from rat lymphocytes, the regulation of gene expression is dependent of various aspects involving cell machinery and transduction signaling pathways. Since enzyme activity cannot be directly correlated to gene expression pattern or to protein levels due to the existence of several post-translational events (Nedeljkovic et al., 2005), these apparently discrepancies between rat and mice gene expression profile in the LPS-induced endotoxemia model still require further investigations.

Adenosine receptors are coupled to G-proteins, and adenosine A2A and A2B receptors in particular can increase intracellular cAMP levels by activating adenylate cyclase. The expression pattern of adenosine receptor subtypes (A1, A2A, A2B, and A3) varies depending on the cell type and pharmacological and biochemical studies have established that A2A receptor is the predominant subtype in immune cells (Huang et al., 1997; Koshiba et al., 1999). The anti-inflammatory effects of extracellular adenosine mediated through adenosine receptor signaling have been known and investigated for a long time (Fredholm et al., 2001). Numerous studies in cellular and animal model systems have provided evidence that A2A signaling pathways are active in limiting inflammation and tissue injury (Hasko and Cronstein, 2004; Linden, 2005; Sitkovsky and Ohta, 2005; Hasko and Pacher, 2008). The interaction between adenosine and A2A receptors is capable of inhibiting inflammation by cAMP induction (Ohta and Sitkovsky, 2009). Experimental data suggest that A2A agonists and antagonists can mediate inflammation by activating and blocking, respectively, an A2A-dependent immunomodulatory mechanism (Ohta and Sitkovsky, 2001). A2A receptors has numerous anti-inflammatory properties, as well as inhibiting T-cell activation (Huang et al., 1997; Erdmann et al., 2005) and limiting the production of inflammatory mediators, such as IL-12, TNF-α and INFγ (Hasko et al., 2000; Pinhal-Enfield et al., 2003; Lappas et al., 2005).

It is important mention that there are some limitations in the current study. Firstly, we have performed these experiments using a single dose of one adenosine A2A agonist. Studies have already shown that other agonists of A2A adenosine receptors present anti-inflammatory actions, such as ATL146e, ATL313, and IB-MECA (Sullivan et al., 2004; Odashima et al., 2005; Lappas et al., 2006; Sevigny et al., 2007). However, few and recent studies have demonstrated that CGS-21680 is able to protect or revert inflammatory states (Lappas et al., 2005; Genovese et al., 2009; Kreckler et al., 2009). The choice of this CGS-21680 dose, which was administered i.p., was based on previous studies indicating its ability to activate adenosine A2A receptor (Martire et al., 2007). We have chosen to test CGS-21680 in two different times of LPS exposures in order to evaluate potential actions of this compound on purine catabolism in modeled endotoxemia. Therefore, testing different CGS-21680 doses and adenosine A2A agonists would reinforce the idea that activation of adenosine A2A receptors may exert a modulatory role in the inflammatory responses. Furthermore, other adenosine A2A antagonists, such as caffeine or ZM241385, can be tested in order to evaluate a possible increase in the severity of the inflammatory state after modeled endotoxemia. We consider that our findings may support future studies aiming the use of different doses of CGS-21680 and different adenosine agonists as anti-inflammatory compounds.

A study performed by Deaglio et al. (2007) provided new information about the mechanism of adenosine generation and immunoregulation by Tregs cells. They demonstrated that Tregs cells express a unique combination of both CD39 (E-NTPDase1) and CD73 (ecto-5′-nucleotidase). These findings demonstrate that the production of adenosine through the enzymatic cascade on the surface of Tregs is important to the A2A-mediated immunosuppressive effects of these cells (Deaglio et al., 2007). These results provide an example of how the coordinate regulation of adenosine production and signaling can impact the immune response. Here we demonstrate that the nucleotide hydrolysis was increased when endotoxemia model was induced in mice. We hypothesized that this increase in ectonucleotidase activities promoted by LPS is a response against the inflammatory process, resulting in ATP depletion and adenosine generation. The effect of LPS on ectonucleotidase activities was prevented when LPS was co-administered with CGS-21680. However, when LPS was administered 24 h before CGS-21680, this compound failed to reverse the effect of LPS. Probably, in the initial phase, when LPS not exert all of inflammatory effects yet, the selective A2A receptor agonist was able to prevent the ectonucleotidase stimulation. In contrast, when the LPS-induced inflammatory process has been already established and the production of adenosine has been enhanced, CGS-21680 was not effective to reverse ectonucleotidase activities to control levels.

5. Conclusion

In summary, our results demonstrate an enhancement of extracellular nucleotide catabolism in lymphocytes after the induction of the modeled endotoxemia, which was prevented, but not reversed by
References