Adenosine is upregulated during peritonitis and is involved in downregulation of inflammation

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Loss of function of the peritoneal membrane is associated with peritonitis. Adenosine levels in sites of inflammation were shown to increase and exhibit immunoregulatory effects. Our aim was to elucidate the regulatory role of adenosine during peritonitis and to test the involvement of peritoneal mesothelial cells (PMC) in adenosine regulation. In a mice model of *Escherichia coli* peritonitis, the adenosine A_{2A} receptor ($A_{2A}R$) agonist (CGS21680) prevented leukocyte recruitment and reduced tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) levels. Peritonitis induced the elevation of adenosine with a peak at 24 h. Analysis of adenosine receptor levels on peritoneum showed that A_1 receptor (A_1R) protein levels peak at 12 h after inoculation and then return to baseline at 24 h, whereas high affinity $A_{2A}R$ protein levels peak at 24 h concomitantly with the peak of adenosine concentration. Low affinity A_{2B} receptor ($A_{2B}R$) levels elevated slowly, remaining elevated up to 48 h. In human PMC (HPMC), the early cytokines, IL-1-α, and TNF-α upregulated the A_{2B} and A_{2A} receptors. However, interferon-γ (IFN-γ) upregulated the $A_{2B}R$ and decreased $A_{2A}R$ levels. Treatment with the $A_{2A}R$ agonist reduced IL-1-dependent IL-6 secretion from HPMC. In conclusion, the kinetics of adenosine receptors suggest that at early stage of peritonitis, the A_1R dominates, and later its dominance is replaced by the G stimulatory (Gs) protein-coupled $A_{2A}R$ that suppresses inflammation. Early proinflammatory cytokines are an inducer of the $A_{2A}R$ and this receptor reduces their production and leukocyte recruitment. Future treatment with adenosine agonists should be considered for attenuating the damage to mesothelium during the course of acute peritonitis.

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The long-term success of peritoneal dialysis is often endangered by structural injury to the peritoneal membrane. This injury is characterized by replacement of the normal peritoneal structure with fibrotic tissue, profusion of capillary vessels, and loss of the mesothelial layer. These morphological alterations are sometimes associated with loss of peritoneal membrane function, leading to the necessity for discontinuation of peritoneal dialysis.[1,2] Apparently, the inflammatory process (which accompanies bacterial peritonitis) is one of the factors which damage the peritoneal membrane. During peritonitis, leukocytes that are shifted into the peritoneal cavity release toxic molecules in order to combat the bacterial invasion. However, these molecules may also be harmful to the peritoneal structure. Moreover, polymorphonuclear neutrophils are recruited into the peritoneal cavity via diapedesis. During this process, these cells strongly adhere to the mesothelium and release proinflammatory compounds such as proteolytic enzymes and reactive oxygen species which possibly injure the peritoneal membrane.[3] Consequently, neutrophil accumulation and their cytotoxic function in the peritoneal exudates must be strictly regulated in order to allow the killing of invading pathogens with minimal damage to the peritoneal tissue.

Peritoneal mesothelial cells (PMC) are not merely bystanders in inflammatory processes, but rather play a central role during inflammation process, within peritoneal cavity.[4] First, as we and others have shown, PMC are a potent source for various proinflammatory mediators, including interleukin (IL)-1α and IL-1β, IL-15, IL-8, monocyte chemotactic protein (MCP)-1, RANTES, IL-6, and growth factors such as transforming growth factor (TGF)-β.[5–9] In addition, PMC constitutively express intracellular adhesion molecule-1,[6] vascular cell adhesion molecule-1, and platelet endothelial cell adhesion molecule-1.[10] PMC are also capable of antigen presentation to lymphocytes and interacting with them.[6] These data underline the role of the mesothelial layer as an important immunomodulator during inflammatory process taking place in the peritoneal cavity.

Over the past few years, a vast number of investigations have reported an involvement of adenosine as an anti-inflammatory mediator.[11,12] Adenosine is an endogenous purine nucleoside that following its release from cells or after

being formed extracellularly diffuses into the cell membrane of surrounding cells where it binds specifically to four known subtypes of adenosine receptors, referred to as A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R.[13–15] All are members of the superfamily of G protein-coupled receptors. The $A_{2A}R$ interacts with the G protein Gs and the ($A_{2B}R$) interacts with the G proteins Gs and Gq (Gq is Gα subunit which stimulates phospholipase C) to stimulate adenylyl cyclase activity thereby causing accumulation of intracellular cAMP levels,[11,16] which have potent immunosuppressive effects.[17] In contrast, the A_1R and A_3R, through interaction with members of the Gi/Go family, inhibit adenylyl cyclase and decrease levels of cAMP.[14] Adenosine has a direct effect on immune cells and contributes to the resolution of inflammation mainly through the $A_{2A}R$.[18–21] By downregulating neutrophil activity and macrophage activation, adenosine inhibits generation of reactive oxygen species and proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), IL-1, IL-6, etc.[12,21–23]

Based on these findings, we hypothesized that adenosine may play an important role in regulating the anti-inflammatory response during acute peritonitis and thus, minimize the peritoneal membrane damage. In order to confirm this hypothesis, we tested the effect of the $A_{2A}R$ agonist on the intraperitoneal influx of leukocytes as well as the proinflammatory cytokine production during peritonitis. We also examined adenosine levels in the peritoneal cavity and the levels and regulation of adenosine receptors on PMC.

RESULTS

$A_{2A}R$ agonist blocks intraperitoneal influx of leukocytes and reduces TNF-α and IL-6 levels

As it has been reported that the $A_{2A}R$ is involved in the anti-inflammatory response,[18–20] we first tested the effect of its specific agonist, CGS-21680 on the intraperitoneal influx of leukocytes during acute peritonitis. As shown in Figure 1, treatment of *Escherichia coli*-inoculated mice with CGS-21680 significantly inhibited the influx of leukocytes into the peritoneal cavity (3.2×10^6 vs 13.2×10^6 cells in control group, $P<0.01$). Similar to this effect, the $A_{2A}R$ agonist strongly reduced TNF-α and IL-6 levels locally and systemically in the infected mice. Levels in the peritoneal fluid (from 48.9 to 22.5 pg/ml, $P>0.05$ and from 101.8 to 20.3, $P<0.05$, respectively) and in the serum (from 471.5 to 182.6 pg/ml, $P<0.001$ and from 150 to 25 pg/ml, $P<0.01$, respectively), as shown in Figure 2.

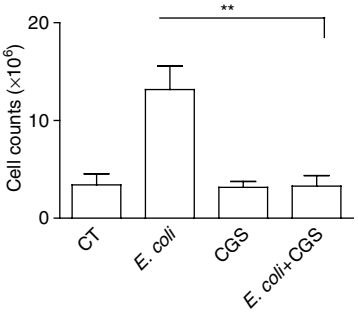

Figure 1 | Effect of adenosine $A_{2A}R$ agonist on leukocyte recruitment in the course of acute peritonitis in mice. CD1 mice were injected with saline or inoculated with a sublethal dose of *E. coli*. At 1 h before inoculation, mice were pretreated with saline or with the $A_{2A}R$ agonist, CGS-21680 (2 mg/kg). Cell exudates were collected at 24 h by peritoneal lavage and counted. The figure is a representative of three similar experiments. Results are presented as mean ± s.e. $N=5$ for each group, $^{**}P<0.01$.

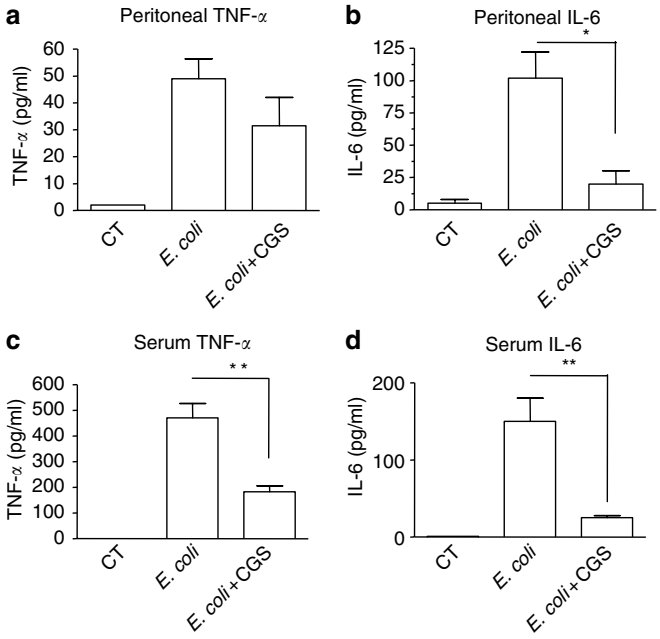

Figure 2 | Effect of $A_{2A}R$ agonist, CGS-21680 on TNF-α and IL-6 levels in the course of *E. coli*-induced peritonitis. CD1 mice were injected with saline or inoculated with a sublethal dose of *E. coli*, and also injected with saline or the $A_{2A}R$ agonist CGS-21680. At 24 h after inoculation, peritoneal lavage was performed, and cell-free supernatants were collected and assayed for (**a**) IL-6, (**b**) TNF-α by enzyme-linked immunosorbent assay kits (R&D Systems). (**c, d**) At 6 or 3 h after inoculation respectively, blood samples from the heart were collected and analyzed for IL-6 and TNF-α, respectively. The figure is a representative of three similar experiments. Results are presented as mean ± s.e. $N=5$ for each group, $^{*}P<0.05$.

Adenosine levels during peritonitis

The next stage was to examine adenosine kinetics during peritonitis in relation to the inflammatory process evolution. At different time points, after intraperitoneal *E. coli* inoculation, peritoneal lavage was performed and serum samples were taken for adenosine determination. No significant changes of adenosine levels were observed in blood following inoculation. Peritoneal leukocytes were counted, and adenosine levels in supernatants were examined concomitantly. As shown in Figure 3a, there is a rapid influx of leukocytes into the peritoneal cavity that reached peak at 12 h, which subsequently recedes slowly over the next 60 h. Adenosine levels rose gradually and reached a maximal level of $2.2 \pm 0.2\ \mu M$ (vs 0.3 ± 0.1) at 24 h and afterwards decreased back to basal levels (Figure 3b). The fact that adenosine levels