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Targeting adenosinergic pathway and adenosine A_{2A} receptor signaling for the treatment of COVID-19: A hypothesis

Mohamed A. Abouelkhair

Department of Biomedical and Diagnostic Sciences, University of Tennessee College of Veterinary Medicine, 2407 River Dr, Knoxville, TN 37996, USA

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ABSTRACT

The most serious health issue today is the rapid outbreak of Coronavirus Disease 2019 (COVID-19). More than 6,973,427 confirmed cases were diagnosed in nearly 213 countries and territories around the world and two international conveyances, causing globally over 400,000 deaths. Epidemiology, risk factors, and clinical characteristics of COVID-19 patients have been identified, but the factors influencing the immune system against COVID-19 have not been well established. Upon infection or cell damage, high amounts of adenosine triphosphate (ATP) are released from damaged cells, which serve as mediators of inflammation through purinergic cell surface receptor signaling. As a protective mechanism to prevent excessive damage to host tissue, adenosine counteracts ATP's effects by adenosine receptor stimulation to suppress the pro-inflammatory response. Adenosine is seen as a major obstacle to the efficacy of immune therapies, and the adenosinergic axis components are critical therapeutic targets for cancer and microbial infections. Pharmacologic inhibitors or antibodies specific to adenosinergic pathway components or adenosine receptors in microbial and tumor therapy have shown efficacy in pre-clinical studies and are entering the clinical arena. In this review, we provide a novel hypothesis explaining the potential for improving the efficiency of innate and adaptive immune systems by targeting adenosinergic pathway components and adenosine A_{2A} receptor signaling for the treatment of COVID-19.

Background

In December 2019, the novel coronavirus (COVID-19 or SARS-CoV-2) emerged as part of a major respiratory disease outbreak centered on Hubei Province, China [1]. More than 6,973,427 confirmed cases were diagnosed in nearly 213 countries and territories around the world and two international conveyances, causing globally over 400,000 deaths [2]. Owing to the genomic similarity of 79% with SARS-CoV, almost the same immune system response is predicted to occur against COVID-19. With the accumulated clinical and experimental data on SARS-CoV, it is possible to hypothesize and even predict how the host immune system can deal with this virus and how it can avoid such host reactions [3,4].

Both the innate and adaptive immune systems are involved in response to SARS-CoV [4]. To defeat the immune response, SARS-CoV applies different mechanisms. One of these mechanisms is through inhibition of interferon type I (IFN-I) expression and signaling [5,6]. Improving extracellular ATP can counteract this inhibition [7].

The cluster of differentiation (CD)73 and CD39 ectonucleotidases on the host cells were found to decrease extracellular adenosine triphosphate (ATP) levels and can rapidly increase adenosine beyond the physiological limit [8]. These elevated concentrations of adenosine exert immunosuppressive action through adenosine A_{2A} and A_{2B} receptors on infiltrating immune cells [9].

In this paper, we intend to provide a new hypothesis to explain how cellular adenosine triphosphate (ATP) can strengthen both innate and cell-mediated immune response to COVID-19. After that, we describe a variety of approaches to target CD73, CD39, and A_{2A}R adenosine signaling that can preserve cellular ATP and block the production and signaling of adenosine.

The hypothesis

Given the crucial role of ATP in IFN-I signaling and production, successive ATP hydrolysis by CD73 and CD39 ectonucleotidases decrease cellular ATP level and make the host cells more vulnerable to viral infections, including COVID-19. Moreover, the elevated concentrations of adenosine, a byproduct of ATP metabolism, exert immunosuppressive action through adenosine A_{2A} and A_{2B} receptors on infiltrating immune cells.

E-mail address: mabouelk@vols.utk.edu.

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Evaluation of the hypothesis

Here, we show how inhibition of components of the adenosinergic pathway and adenosine signaling via $A_{2A}R$ can preserve extracellular ATP, counteract with COVID-19 defensive mechanisms, and rejuvenate the innate and adaptive immune response.

ATP limits viral replication by facilitating IFN-β production and signaling

SARS-CoV and COVID-19 impair the rapid increase of the IFN-I, which deactivates the so-called "initial alarm" of the innate immune system and promotes virus replication [4]. Such fast coronavirus replication kinetics and relative delay in IFN-I signaling orchestrate the induction of an inappropriate inflammatory response during SARS-CoV infection, extensive vascular leakage, impaired virus-specific T-cell responses and consequent immunopathology of the lungs [10]. Zhang et al. have shown that improving extracellular ATP can counteract this inhibition by facilitating the secretion of IFN-I through the signaling pathway P38/JNK/ATF-2 [7]. Consequently, ATP-depleted cells are more vulnerable to viral infections such as SARS-CoV and probably COVID-19 [11].

Kumari G et al. found that SARS-CoV-2 is more susceptible to IFN-I pretreatment than SARS-CoV and type I IFN can be useful for SARS-CoV-2 treatment if appropriate parameters are determined [12]. Fundamental changes occur in the immune cells after IFN-I secretion, which turns them into the so-called antiviral state [13]. Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is one of the signaling pathways that participate in this process [14,15]. JAKs are ATP-dependent enzymes which are bound to cytoplasmic regions of type I and II cytokine receptors. Upon IFN-I stimulation, JAKs are activated and phosphorylate STATs, which results in dimerization and translocation of STATs to the nucleus [15]. Cellular ATP depletion interferes with IFN-I signaling and thus impairs the transformation into "antiviral state" [4].

Ectonucleotidases and adenosine receptor activity promote immunosuppression

Nonetheless, crucial mechanisms underlying lung injury and dysfunction in COVID-19 or any other pulmonary viral infection are currently poorly described. It was hypothesized that influenza A virus induces *de novo* ATP synthesis and release from infected Alveolar epithelial type II (ATII) cells. The released ATP could be rapidly metabolized to adenosine at an accelerated rate (due to increased ectonucleotidase CD73 activity), which plays a pivotal role in influenza lung injury due to its impact on adenosine receptors [16].

Successive ATP processing by CD73 and CD39 ectonucleotidases decreases cellular ATP levels and rapidly increases adenosine from a low homeostatic level (20–200 nM) to as much as 1,000–10,000 nM [8]. These elevated concentrations of adenosine exert immunosuppressive action through adenosine A_{2A} and A_{2B} receptors on infiltrating lymphocytes, NK cells, and macrophages [9].

Practical approaches to target the adenosinergic pathway and adenosine A_{2A} receptor signaling

CD39 inhibits the immune system by degrading ATP into AMP, which is then further degraded into adenosine by CD73. In the last decade, CD73, CD39, and $A_{2A}R$ receptors' potential as immunotherapy targets for cancer and microbial infections have rapidly increased [17–22]. Humanized monoclonal anti-CD39, such as IPH5201 (Innate Pharma), have been developed [23]. Such antibodies bind to CD39 upon administration and prevent CD39-mediated conversion of extracellular ATP to AMP. Targeting CD39 by blocking antibodies or inhibitors such as POM-1, was found to enhance T cells and NK cells' functionality, as well as decreased Treg-mediated suppression of T cell proliferation [23,24].

Indeed, targeting CD39 is useful to curb ATP depletion, but to reduce adenosine accumulation, CD73 should also be targeted. Large numbers of studies on biological models as well as the constant publication of CD73 enzyme inhibitors demonstrates an interest in inhibiting CD73 in clinics. Monoclonal anti-CD73 antibody BMS-986179 displayed possible immunomodulatory activity [19]. Anti-CD73 monoclonal antibody targets and binds to CD73 upon administration, leading to clustering and internalization of CD73 [25]. Such binding prevents CD73-mediated conversion of extracellular adenosine monophosphate (AMP) to adenosine and reduces free adenosine, which blocks adenosine-mediated suppression of lymphocyte activity and increases CD8-positive cell function. It also stimulates macrophages, suppressing both myeloid-derived suppressor cells (MDSCs) and regulatory T lymphocytes.

Small-molecule CD73 inhibitor, such as AB680 (Arcus Biosciences) [26]; benzothiadiazine derivatives (GlaxoSmithKline) [27], inhibit the enzymatic activity of CD73. AB680 is a highly potent, reversible, and selective small-molecule CD73 inhibitor [26]. In the presence of high AMP concentrations, AB680 robustly restored IFN-γ production and proliferation of human $CD4^+$ and $CD8^+$ T cells. AB680 is currently in preclinical development as a potential anti-tumor agent. AB680 provides differential benefits relative to monoclonal antibodies, such as greater inhibition of CD73 enzymatic activity (both soluble and cell-bound) and deeper penetration of target sites.

CD73 small interfering ribonucleic acid (siRNA) molecules represent a promising tool for CD73 gene expression inhibition. A previous study showed that treatment with nanoemulsion-CD73 siRNA complexes decreased tumor CD73 expression, AMPase activity, adenosine production and reduced tumor growth by 60% in a preclinical model of glioblastoma [28].

Collectively, pharmacologic inhibitors or antibodies to CD39 and CD73 ectonucleotidases may potentially have preventive effects through the protection of extracellular ATP from hydrolysis and production of immunosuppressive molecule, adenosine, and maintaining the ATP level for activating the initial IFN-I secretion and signaling as "initial alarm" of the innate immune system (Fig. 1).

Adenosine A_{2A} receptor antagonists, for example, istradefylline and Ciforadenant, binds to adenosine A_{2A} receptors on the surface of the immune cells such as T-lymphocytes, natural killer cells (NK), macrophages, and dendritic cells (DCs) [20,29]. A_{2A} receptor antagonists prevent adenosine from interacting with the A_{2A} receptors of these primary immune surveillance cells, thus eliminating the immunosuppression. Ciforadenant (formerly CPI-444), an oral $A_{2A}R$ antagonist, suppresses the expression of several checkpoint pathways on CD8 + effector T cells and CD4 + FoxP3 + Tregs and also have profound effects in restoring immunity at draining lymph nodes by decreasing the expression of programmed cell death (PD-1) and lymphocyte-activation gene 3 (LAG-3) [30].

The therapeutic gain of targeting multiple components within the adenosinergic pathway is much higher than one. Simultaneous administration of an anti-CD73 monoclonal antibody and an $A_{2A}R$ antagonist demonstrated synergy for tumor metastasis control because it inhibits the compensatory response of $A_{2A}R$ blockade to increase CD73.

Consequences of the hypothesis

Given these considerations, a novel hypothesis is proposed, explaining the potential for improving the efficiency of innate and adaptive immune response for COVID-19 prevention and control by blocking adenosine production via CD73 and signaling via $A_{2A}R$. The cellular ATP level can theoretically be considered a key component in COVID-19 infectivity and prognosis. Enhancing cellular ATP, through targeting CD39, is predicted to strengthen the innate immune response against SARS-CoV-2. This hypothesis will serve as a catalyst for further

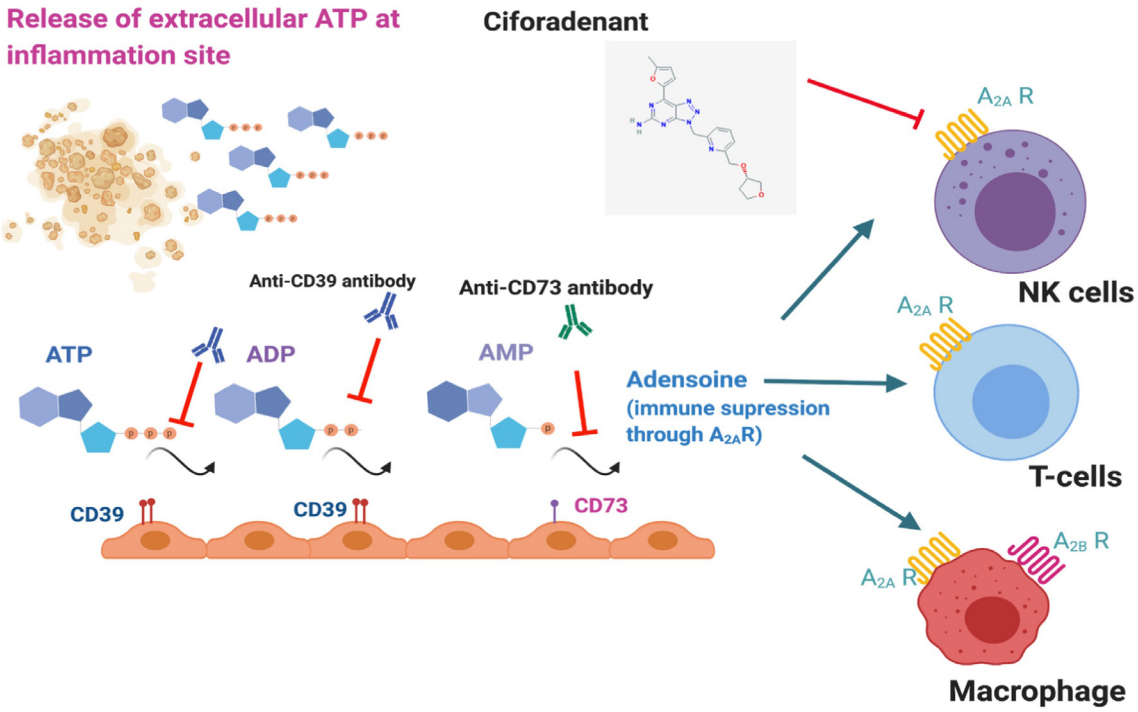

Fig. 1. Targeting the adenosinergic pathway components by using anti-CD73, anti-CD39 monoclonal antibodies and $A_{2A}R$ receptor antagonist.

research into this issue.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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