Targeting the CD73-adenosine axis in COVID-19 immunotherapy

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25 **Abstract**

26 The most serious health issue today is the rapid outbreak of Coronavirus Disease 2019 (COVID-
27 19 or SARS-CoV-2). It is now a global pandemic and is a huge concern for public health. So far,
28 more than 3,500,000 confirmed cases were diagnosed in nearly 212 countries and territories
29 around the world and 2 international conveyances, causing globally over 250,000 deaths.
30 Epidemiology, risk factors, and clinical characteristics of COVID-19 patients have been
31 identified but the factors influencing the immune system against COVID-19 have not been well
32 established. Upon infection or cell damage, high amounts of adenosine triphosphate (ATP) are
33 released from damaged cells, which serve as mediators of inflammation through purinergic
34 cell surface receptor signaling. As a protective mechanism to prevent excessive damage to host
35 tissue, adenosine counteracts ATP's effects by adenosine receptor stimulation to suppress the
36 pro-inflammatory response. Currently, the adenosine pathway is seen as a major obstacle to the
37 efficacy of immune therapies and is an important therapeutic target for cancer and microbial
38 infections. Pharmacologic inhibitors or antibodies specific to adenosine pathway components or
39 adenosine receptors in microbial and tumor therapy have shown efficacy in pre-clinical studies
40 and are entering the clinical arena. In this review, I propose a novel hypothesis explaining the
41 potential for improving the efficiency of innate and adaptive immune systems by co-inhibition of
42 CD73 and A_{2A}R adenosine Signaling for COVID-19 prevention and control.

43 **Introduction**

44 In December 2019, the novel coronavirus (COVID-19 or SARS-CoV-2) emerged as part of a
45 major respiratory disease outbreak centered on Hubei Province, China (Gralinski and

Menachery, 2020). It is now a global pandemic and is a huge concern for public health. So far, more than 3,500,000 confirmed cases were diagnosed in nearly 212 countries and territories around the world and 2 international conveyances, causing globally over 250,000 deaths (Worldometer, 2020, May 02). Taxonomically, SARS-CoV-2 is a Betacoronavirus (lineage B) closely linked to SARS-CoV and SARS-related bat coronaviruses (Jaimes et al., 2020). Thereafter, beta coronaviruses from pangolins were described as close relatives to SARS-CoV-2 (Jaimes et al., 2020). 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 particular virus and how the virus can avoid such host reactions (Lu et al., 2020; Taghizadeh-Hesary and Akbari, 2020). The most common symptoms of COVID-19 are fever, fatigue, and respiratory symptoms such as cough, sore throat, and acute respiratory distress syndrome (ARDS). Although diarrhea was reported in approximately 20-25% of patients with SARS-CoV, intestinal symptoms were rarely reported in patients with COVID-19 (Huang et al., 2020; Wu et al., 2020). Both the innate and adaptive immune systems are involved in response to SARS-CoV. In order to defeat the immune response, SARS-CoV applies different mechanisms. One of these mechanisms is through inhibition of interferon type 1 (IFN-1) expression and signaling (de Wit et al., 2016; Kindler and Thiel, 2016). IFNs can be divided into three subtypes: type I (α and β), type II (γ), and type III (λ) (Parkin and Cohen, 2001; Akira et al., 2006; Zhang et al., 2017b). Type I IFNs are evolutionarily preserved

and consist of multiple IFN- α subtypes (14 in human, 11 in mouse) and single subtypes β , ϵ , τ , ω , ζ , and κ , all of which are essential in the fight against RNA and DNA virus infection (Chen et al., 2004). IFN-I are all recognized by interferon- α/β receptor (IFNAR), which results in the activation of multiple signaling effectors and antiviral response pathways that coordinate invoking gene and protein regulation including IFN-stimulated protein of 15 kDa (ISG15), the myxovirus resistance 1 (Mx1), ribonuclease L (RNaseL) and protein kinase R in the target cells, all of them exert potential direct antiviral effects (Zhao et al., 2005; Sadler and Williams, 2008). In the meantime, inducible type I IFN plays a key role in antiviral immune responses by inducing cellular resistance to viral infection and promoting apoptosis of virus-infected cells (Zhao et al., 2005; Taghizadeh-Hesary and Akbari, 2020).

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

The various functions of living organisms are regulated by extracellular signaling of purines and pyrimidine derivatives, including adenosine triphosphate (ATP) (Abbracchio and Burnstock, 1998), adenosine diphosphate (ADP) (Burnstock, 2006) and adenosine (Zimmermann, 1992). Such molecules are produced via the release from cells or the metabolic hydrolysis of their precursors in the extracellular space. The physiological roles of these nucleotides and nucleosides had not been investigated until the 1970s. The focus of the research was limited to the synthesis of ATP and its energy role. ATP plays a primary role in stimulating the inflammatory response via the purinergic pathway using P2Y receptors (P2YRs) (Jacob et al., 2013). ATP can be highly released by virus-infected cells during the early stages of infection, which is different from pathogen-induced cytokines

(Taghizadeh-Hesary and Akbari, 2020). Thus, the released ATP could serve as a danger signal to alarm the immune system to fight against invading pathogens.

SARS-CoV impairs the rapid increase of the IFN-I. As a result, the so-called "initial alarm" of the innate immune system is deactivated by unknown mechanisms (Taghizadeh-Hesary and Akbari, 2020). This promotes coronavirus replication (Zhang et al., 2017a). Such rapid SARS-CoV replication kinetics and relative delay in IFN-I signaling orchestrate 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 in infected BALB/c mice (Channappanavar et al., 2016). Such results are similar findings in SARS patients (Nicholls et al., 2003; Channappanavar et al., 2016). Therefore, not only the magnitude of the response but also its timing may seem to play a crucial role in the infection with coronavirus (**Figure 1**).

Zhang et al. have shown that improving extracellular ATP can reverse this cycle (Zhang et al., 2017a) by facilitating the secretion of IFN through the signaling pathway P38/JNK/ATF-2 (Zhang et al., 2017a). Consequently, ATP-depleted cells are more vulnerable to viral infections such as SAR-CoV and probably COVID-19. (Johnson et al., 2019).

Fortunately, 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 (Lokugamage et al., 2020).

Fundamental changes occur in the immune cells after IFN-I secretion, which turns them into the so-called antiviral state (Parkin and Cohen, 2001). Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is one of the signaling pathways that participate in this

process (Renauld, 2003; Seif et al., 2017). 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 (Seif et al., 2017). Cellular ATP depletion obviously interferes with IFN-I signaling and thus impairs the transformation into "antiviral state" (Taghizadeh-Hesary and Akbari, 2020).

CD73 and Adenosine Receptor Activity Promote Immunosuppression

Nucleotides and adenosine are metabolized by various enzymes in the extracellular space. These nucleotide-metabolizing enzymes, collectively called ecto-nucleotidases, have potential as drug targets (Zimmermann et al., 2012). Nucleotides such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP) are extruded from damaged and necrotic cells at inflammation sites and hydrolyzed by CD39 into adenosine monophosphate (AMP) (Perrot et al., 2019). AMP is then hydrolyzed to adenosine by CD73 (Perrot et al., 2019). Successive ATP processing by CD73 and CD39 ectonucleotidases decreases cellular ATP levels and can rapidly increase adenosine from a low homeostatic level (20-200 nM) to as much as 1,000-10,000 nM (Abdel-Magid, 2017). These elevated concentrations of adenosine exert immunosuppressive action through adenosine A_{2A} and A_{2B} receptors on infiltrating lymphocytes, shielding the cells from excessive inflammatory reactions and thus providing a self-limiting mechanism to resolve the immune response (Ohta and Sitkovsky, 2001).

Nonetheless, crucial mechanisms underlying lung injury and dysfunction in COVID-19 or any other pulmonary viral infection are currently poorly described. Influenza virus infection of BALB/c mice induces increased channel-mediated release of nucleotides UTP and ATP into bronchoalveolar lining fluid (BALF) (Aeffner et al., 2012), which contributes to lung edema and

hypoxemia. It was hypothesized that influenza induces de novo ATP synthesis and release from infected Alveolar epithelial type II (ATII) cells. The released ATP in BALF 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 receptor (Wolk et al., 2008).

Targeting the CD73-adenosine axis may potentially help for the prevention and control of COVID-19

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 (**Figure 2**).

In the last decade, the potential of CD73 and A_{2A}R antagonist as immunotherapy targets have rapidly increased (Willingham et al.; Siu et al., 2018; Powderly et al., 2019; Harvey et al., 2020). Adenosine A_{2A} receptor antagonist, 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) (Willingham et al.; Antonioli et al., 2008). A_{2A} receptor antagonist prevents 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 antitumor immunity at draining lymph nodes by decreasing the expression of programmed cell death (PD-1) and lymphocyte-activation gene 3 (LAG-3) (Leone et al., 2015). Monoclonal anti-CD73 antibody BMS-986179 displayed possible immunomodulatory and antineoplastic activity (Siu et al., 2018). Anti-CD73 monoclonal antibody targets and binds to CD73 upon administration, leading to clustering and internalization of CD73 (Terp et al., 2013). This prevents CD73-mediated conversion of extracellular adenosine monophosphate (AMP) to adenosine and decreases free adenosine which blocks adenosine-mediated suppression of lymphocyte activity and increases CD8-positive cell function. This also stimulates macrophages, suppressing both myeloid-derived suppressor cells (MDSCs) and regulatory T lymphocytes.

Conclusions

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 the production of adenosine 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 CD73-adenosine axis is predicted to strengthen both innate and cell-mediated immune systems. The therapeutic gain of targeting both components of the adenosine pathway is much higher than one because it inhibits the compensatory response of A_{2A}R blockade to increase CD73. This indicates the potential benefits of integrating adenosine-related therapeutic approaches with existing clinically approved antiviral drugs.

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Figure 1: Kinetics and the magnitude of the IFN-1 response are crucial in infection with coronavirus. Relative delay in IFN-I signaling orchestrates the induction of an inappropriate inflammatory response and consequent lung immunopathology.

Figure 2: Targeting the CD73-adenosine axis by using anti-CD73 monoclonal antibodies and A_{2A}R receptor antagonist.