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REGULATION OF THE T-CELL RESPONSE BY CD39

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Abstract

The ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, or CD39) catalyzes the phosphohydrolysis of extracellular adenosine triphosphate (eATP) and diphosphate (eADP) released under conditions of inflammatory stress and cell injury. CD39 generates adenosine monophosphate (AMP), which is in turn used by the ecto-5'-nucleotidase CD73 to synthesize adenosine. These ectonucleotidases have major impacts on the dynamic equilibrium of pro-inflammatory eATP and ADP nucleotides vs. immunosuppressive adenosine nucleosides. Indeed, CD39 plays a dominant role in the purinergic regulation of inflammation and the immune response because its expression is influenced by genetic and environmental factors. Here, we review the specific role of CD39 in the kinetic regulation of cellular immune responses in the evolution of disease. We focus on the effects of CD39 on T cells and explore potential clinical applications in autoimmunity, chronic infections and cancer.

Purinergic signaling – the basics

Adenosine 5'-triphosphate (ATP) and diphosphate are nucleoside tri and di-phosphates that play important pathophysiological roles. Intracellular ATP is the main energy currency in the cell, participating in virtually all biological processes. ATP and other nucleotides are released to the extracellular space under basal, quiescent or activated conditions such as during cell death or apoptosis. Extracellular ATP (eATP) and related derivatives play important functions as signaling molecules, participating in both autocrine and paracrine

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Conflict of interest

The authors state no conflict of interest.

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circuits. These circuits regulate cellular metabolism, migration, proliferation and apoptosis through signaling pathways triggered by type 2 purinergic/pyrimidinergic P2Y and P2X receptors (1, 2).

Purinergic signaling is an important regulator of the immune response. Indeed, eATP is usually a strong pro-inflammatory signal, while extracellular adenosine produced from eATP degradation is mainly immunosuppressive (Fig. 1). Thus, it is not surprising that eATP levels and biological effects are tightly regulated by the catalytic effects of ecto-enzymes expressed on the plasma membrane of immune cells.

In this context there are major groups of ectonucleotidase families discovered, then cloned and functionally characterized using pharmacological agents and other means. These families include alkaline phosphatases, ectonucleoside triphosphate diphosphohydrolases (E-NTPDase) and the ecto-nucleotide pyrophosphatases/ phosphodiesterases (ENPP). Other ecto-enzymes involved in the catalysis of extracellular nucleotides and nucleosides include ecto-5'-nucleotidase CD73, adenosine deaminases and NAD glycohydrolases, CD38/NADase, dinucleoside polyphosphate hydrolases, adenylate kinase, nucleoside diphosphate kinase, and potentially ecto-F1-Fo ATP synthases (reviewed in (3)).

This review focuses on the biology of two ectonucleotidases, which play central immunoregulatory roles: CD39, the prototypic member of the ENTPD family which degrades eATP into adenosine monophosphate (AMP) and ecto-5'-nucleotidase CD73, which degrades eAMP into adenosine (1). Recent work has shown that ENPP1, also known as CD203a, can generate eAMP from the metabolism of NAD and ADP-ribose (4). In this manuscript, however, we will examine the specific role of CD39 in generating eAMP and note the associated links to CD73 in the regulation of the cellular immune response, with a focus on its direct and indirect effects of modulated purinergic signaling on T cells.

ATP release

ATP is almost exclusively present inside cells, where it is found at high levels with concentrations ranging from 1 to 10mM (5). In the extracellular milieu, ATP concentrations are significantly lower, in the nanomolar range (5). ATP is released into the extracellular space during necrosis and apoptosis (6, 7). In addition, inflammatory cells and platelets also release eATP during their activation in the context of inflammation, ischemia, hypoxia and the response to pathogens (6).

Different mechanisms mediate the controlled release of ATP to the extracellular medium. Pannexins are conserved transmembrane channels that allow the passage of ions and small molecules. Pannexin 1 channels participate in the release of ATP from activated T cells and dendritic cells (DCs) to the extracellular medium (2, 8–10). Pannexins have also been linked to ATP release during apoptosis (11, 12). In addition, the gap junction hemichannel connexin 43 is described to mediate ATP release by glial and endothelial cells during inflammation (13–15). Finally, human B cells activated via the B cell receptor (BCR) and toll-like receptors can release ATP stored in Ca²⁺-sensitive secretory vesicles (16).

The commensal flora is recognized to play an important role in the control of the immune response in the context of allergy, autoimmunity and cancer (17–20). Different molecules mediate the effects of the commensal flora on the immune response, including long-chain fatty acids and tryptophan derivatives (21–23). It has been recently shown that eATP produced by commensal bacteria activates purinergic signaling to promote the differentiation of intestinal T helper 17 cells (Th17) (24). Conversely, several pathogens express ectonucleotidases that may modulate the immune response through their effects on purinergic signaling (25). Thus, collectively, these findings point to different cell- and stimulus-specific mechanisms of eATP release. Moreover, these findings suggest that eATP produced by microorganisms and by host cells in response to microbial molecules such as TLR agonists plays an important role in the relationship between the host and the commensal flora (26).

eATP signaling

eATP activates P2 purinergic/pyrimidinergic receptors expressed on the surface of virtually all mammalian cells. Two families of P2 receptors have been identified, which differ in protein structure and function: inotropic P2X and metabotropic P2Y (Table 1).

P2X receptors are oligomeric ion channels that present two transmembrane domains and an extracellular loop that binds eATP. When activated, P2X receptors promote the influx/efflux of cations and the activation of Ca^{2+} and K^{+} dependent signaling pathways. Notably, P2X receptors are only activated by eATP. Seven subtypes of P2X receptors have been identified (P2X1-P2X7), the P2X7 receptor (P2rX7) is the one that has been most studied for its role in the immune response.

P2Y receptors are G protein-coupled receptors (GPCRs) that present seven transmembrane domains, and are activated by ATP and also by other nucleotides such as ADP, UTP, UDP and UDP-glucose. Eight subtypes of human P2Y receptors have been identified (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14), which associate with different G proteins to trigger the activation of distinct signaling pathways. P2Y1, 2, 4, 6 and 11 receptors are coupled to G_q/G_{11} that activate the phospholipase C (PLC), increasing the intracellular levels of inositol-1,4,5-triphosphate (IP_3) and diacyl glycerol (DAG) and leading to the release of calcium from the endoplasmic reticulum. P2Y12, 13 and 14 receptors are coupled to $\text{G}_{i/o}$ proteins, which mainly inhibit adenylate cyclase, leading to the decrease of cyclic adenosine monophosphate (cAMP) intracellular levels and the modulation of ion channel activity (27).

P2X and P2Y receptors are both expressed on immune cells, including T and B cells, as well as DCs, macrophages and neutrophils. However, it is unclear which factors determine the signaling pathway activated by eATP in each cell type. One factor that may be involved is the local concentration of eATP, because P2X receptors are usually activated by lower eATP concentrations than P2Y receptors. An additional factor to consider is the distance of the P2 receptors to the channels, vesicles or cells that release eATP, because eATP is quickly transformed into other metabolites by cell surface ectonucleotidases. For example, in T cells

and neutrophils, pannexins 1 are localized close to the P2X7 and P2Y2 receptors, which are therefore activated in an autocrine manner when ATP is released (9).

Regulation of eATP levels

The half-life of eATP is relatively short as a result of its rapid scavenging and phosphohydrolysis to AMP and adenosine by the combined activity of ectonucleotidases. Thus, considering the pro-inflammatory properties of eATP and the anti-inflammatory activity of adenosine, various families of ectonucleotidases as noted above play important roles in immune regulation (28). In this review, we focus on the ENTPD prototype family member CD39, as well as examining functional links with CD73 in the generation of adenosine.

The ENTPD family also comprises eight members, of which four (NTPDase1, 2, 3, and 8) are expressed on the cell surface and in general hydrolyze ATP or ADP, ultimately to AMP. ENTPD2 is however a preferential ecto-ATPase (29).

ENTPD1 (CD39) is found on monocytes, DCs, neutrophils, B lymphocytes and on some subsets of NK and T cells. Because of its ability to hydrolyze pro-inflammatory eATP and promote the synthesis of immuno-suppressive adenosine (in tandem with CD73), CD39 has important roles in limiting inflammation (Table 2).

With respect to the kinetics of phosphohydrolysis, there is feed forward inhibition of CD73 by ADP; hence CD39 is the rate limiting ectonucleotidase in the blood and extracellular environment (30). CD39 biological activity is substantively compromised by acute inflammatory stress and reactive oxygen species; as in ischemia reperfusion responses (31, 32). The acute inflammatory loss of vascular NTPDase activity is therefore highly pertinent to the delayed evolution of adenosinergic responses, which allows platelet activation to occur and acute inflammatory “danger” responses to proceed, while compartmentalizing inflammatory responses, removing pathogenic organisms.

Transcriptional induction of CD39 and CD73 ectonucleotidases and increased immune cell infiltration at sites of injury results in conversion of a dominant P2-environment to one associated with decreases in the levels of nucleotides and predominant adenosinergic responses. It should be also noted that extracellular nucleotides may further serve as negative modulators of immunity, or as immunosuppressants. Indeed, chronic and repetitive exposure to low extracellular nucleotide levels tends to suppress immunity and inflammation (33).

In the following sections, we will discuss the different roles played by CD39 and these kinetic fluxes in nucleotides and pathways of adenosine generation in the regulation of the T-cell response (Fig. 2).

Effects of eATP and CD39 on T cells

eATP boosts T-cell activation

Following the engagement of the T-cell receptor (TCR), pannexin 1 channels localize to the immune synapse together with P2X receptors, resulting in the release of ATP that activates

the P2X receptors, triggers calcium influx and boost cell activation (9). Thus, eATP released during T-cell activation acts in an autocrine manner to amplify TCR-triggered activation and enhance IL-2 production (9, 34). In addition, the eATP released during T-cell activation can also act as a paracrine messenger, inducing a P2rX4/P2rX7-dependent influx of calcium in neighboring lymphocytes that results in the reduction of T-cell motility in the lymph node and favors interactions between T cells and antigen presenting cells (35).

It has to be noted, however, that the P2rX7-dependent effects of eATP on T cells are concentration-dependent: low eATP concentrations trigger P2X receptor responses (around 250 nM), while high eATP concentrations (1mM) and prolonged P2rX7 stimulation induce pore formation and apoptosis (36, 37).

CD39 stabilizes FoxP3⁺ Tregs and contributes to their suppressive function

Specialized T-cell populations limit the activity of self-reactive elements in the immune system to prevent the development of immune pathology. One of these populations is constituted by CD4⁺ regulatory T cells, further characterized by the expression of the transcription factor FoxP3 (FoxP3⁺ Tregs). Several mechanisms have been described to mediate the immunoregulatory effects of FoxP3⁺ Tregs, among them are the expression of surface molecules such as Lag-3 and CTLA4, and the production of immunosuppressive cytokines such as IL-10 and TGF- β (38–40).

CD39 and eATP, however, also play a significant role in the suppressive function and stability of FoxP3⁺ Tregs. CD39 and CD73 are co-expressed on the surface of human and murine regulatory FoxP3⁺ Tregs (41, 42). In cooperation with CD73, CD39 contributes to FoxP3⁺ Treg suppressive activity by promoting the generation of adenosine, which acts directly on T cells to modulate their activation through signaling cascades controlled by the A2A adenosine receptor (41, 42). In addition, adenosine-triggered increments in cAMP intracellular levels may result in the transactivation of the *Entpd1* promoter, increasing CD39 expression by FoxP3⁺ Tregs (43).

CD39 also contributes to FoxP3⁺ Treg stability. eATP signaling through P2rX7 and pErk interferes with both the differentiation and suppressive function of FoxP3⁺ Tregs (44). Interestingly, the decrease in the differentiation and suppressive function of FoxP3⁺ Tregs triggered by eATP is linked to decreased FoxP3 expression and stability. Considering that FoxP3 is needed not only to initiate, but also to maintain the transcriptional program of FoxP3⁺ Tregs, direct effects of eATP on FoxP3 stability are likely to mediate these effects through different mechanisms.

The transcription factor HIF-1 α has been shown to interfere with FoxP3⁺ Treg differentiation by triggering FoxP3 ubiquitination and degradation (45). HIF-1 α mediates the cellular response to hypoxia (46, 47), however additional stimuli can also result in the activation of HIF-1 α dependent responses. eATP is reported to induce the expression of HIF-1 α responsive genes through a P2rX7-dependent pathway (48, 49). Hence, the suppressive effects of eATP on FoxP3⁺ Tregs likely involve the HIF-1 α dependent ubiquitination and degradation of Foxp3. In this context, CD39 expression may stabilize FoxP3⁺ Tregs through its ability to deplete eATP and limit HIF-1 α activation. Moreover, the

stabilization of FoxP3⁺ Tregs by CD39 would be particularly advantageous in the microenvironment of inflammatory sites, which are characterized by high levels of eATP (2, 27). In support of this hypothesis, recent genetic analyses support the control of the peripheral FoxP3⁺ Tregs pool in humans by CD39 (50). Hypoxia and adenosine may also affect CD39 expression through additional mechanisms (43, 51). Collectively, these data show that CD39 contributes to FoxP3⁺ Treg dependent immune modulation through the synthesis of adenosine in cooperation with CD73, the stabilization of FoxP3 and the arrest of eATP-triggered T-cell apoptosis to which FoxP3⁺ Tregs show increased sensitivity (52).

CD39 boost Tr1 cell differentiation and function

Type 1 regulatory T (Tr1) cells are characterized by the production of IL-10 and the lack of FoxP3 expression, initially described by Roncarolo and co-workers (53). Tr1 cells have non-redundant roles in limiting inflammation, enforcing immune tolerance in diverse contexts ranging from HLA-mismatched fetal liver hematopoietic stem cell transplants to autoimmune diabetes (54–56). Indeed, Tr1 cell deficits have been described in autoimmune diseases such as multiple sclerosis (MS) (57). IL-27 is a potent inducer of Tr1 cell differentiation (58–60). Indeed, we found that IL-27 promotes Tr1 cell differentiation through signaling pathways mediated by AHR and other molecules (61–64).

We recently found that AHR in combination with STAT3 promotes the expression of CD39 in IL-27 induced Tr1 cells (65) (Fig. 3). Similarly to what has been shown for FoxP3⁺ Tregs, CD39 contributes to both, Tr1 cell differentiation and function. CD39 contributes to the suppressive function of Tr1 cells *in vivo* and *in vitro*. However, Tr1 cells induced with IL-27 do not express CD73. Accordingly, the CD39-dependent suppressive activity of Tr1 cells requires the synthesis of adenosine in cooperation with CD73 expressed by effector T cells and DCs. This observation suggests that the cooperative generation of adenosine by CD39 in Tr1 cells and CD73 in other immune cells may limit adenosine production, minimizing fibrosis and other side effects of chronic adenosinergic signaling (66).

CD39 also boosts the differentiation of Tr1 cells. Indeed, CD39 deficiency results in decreased Tr1 cell differentiation in response to IL-27. As we already mentioned, AHR plays a central role in the transcriptional programs that drive Tr1 cell differentiation in response to IL-27 (63–65, 67). AHR and HIF-1 α associate with ARNT to control the expression of their target genes (46, 68). In the absence of CD39, increased eATP levels result in HIF-1 α activation. This in turn displaces AHR from its interaction with ARNT and promotes AHR ubiquitination and degradation suppressing AHR-dependent signaling (65). Therefore, CD39 promotes Tr1 cell differentiation by decreasing eATP levels, limiting P2rX7-dependent purinergic signaling.

AHR directly promotes CD39 expression in Tr1 cells. Thus, it is tempting to speculate that molecules that increase AHR expression or activity, such as melatonin (62) or AhR agonists (68) might be exploited to increase CD39 expression in Tr1 cells and consequently, the suppressive function and stability. Together with specific P2rX7 inhibitors (69), these molecules might offer new avenues for the development of Tr1 cell-based immunotherapies.

CD39 expression endows effector T cells with suppressive activity

Th17 cell subpopulations that differ in their pathogenicity are differentiated based on the expression of specific transcriptional programs and effector molecules (70–73). GM-CSF, for example, is expressed by pathogenic Th17 cells (74, 75). Conversely, the production of IL-10 has been linked to non pathogenic Th17 cells that might be endowed with suppressive activity (71, 72). Non-pathogenic Th17 cells generated with IL-6 and TGF- β also express CD39 and CD73 (76), and have also been identified in humans (77). Indeed, CD39 is reported to mediate the suppressive activity of non-pathogenic Th17 cells, interfering for example with tumor-specific immunity through an adenosine-dependent mechanism.

During chronic immune responses to tumors or infections effector T cells gradually lose effector functions while they acquire the expression of multiple inhibitory molecules. Exhausted T cells might even gain the ability to actively suppress other effector T cells (78). This process has been named T-cell exhaustion, but the molecular mechanisms that control it are not completely understood. It has been recently reported that CD39 expression is associated with the exhausted immune cell phenotype in cancer and chronic infection (79). It is not clear yet whether CD73 expression is also a common feature shared by the exhausted T cells induced in different physiologic scenarios. However, collectively these findings suggest that the expression of CD39 may endow effector T cells with regulatory functions.

Subpopulations of long-lived T memory-type cells also express CD39, which may well impact cellular metabolic profiles and promote survival. The mechanisms that control the longevity of immune cells and mediates their resistance to cell death under conditions of stress or activation-induced cell death (AICD) remain unclear. Work in mammalian cell systems, insects, worms and yeast suggest that insulin/IGF signaling, AMP-kinase (AMPK)-FOXO transcription factors and mammalian target of rapamycin (mTOR) all regulate stress resistance and organism longevity (80). Interestingly, eATP/CD39 can modulate mTOR activation (49), and it is entirely feasible that regulation of purinergic signaling will affect T-cell survival.

These observations suggest that specific mechanisms operate to modulate purinergic responses in memory T cells. The response of memory T cells to adenosine and specific receptor agonists might be modulated at the level of intracellular cyclic-AMP and the signaling pathways it controls. Hence, any potential resistance of T memory or other immune cells to the effects of adenosinergic signaling could be associated with the upregulation of immune cell phosphodiesterases (PDE) (81).

In addition, it is also feasible that following prior exposure to adenosine, PKA-mediated desensitization changes responses triggered by A2A or other receptors. G protein-coupled receptor kinases (GRKs) are also thought to be important in mediating the agonist-induced phosphorylation and consequent desensitization of purinergic responses. It is also possible that a differentially spliced A2A receptor is expressed, preferentially internalized or shed in long-lived T cells. The native A2A receptor has a long C-terminus (of >120 residues), which clearly binds to adenylate cyclase (AC) but was originally viewed as the docking site for kinases and the beta-arrestin family to initiate receptor desensitization and endocytosis. Hence, the truncation of A2A C-terminus as a result of alternative splicing (82, 83), would

preclude AC-dependent cAMP responses while maintaining signaling via MAP kinases. Alternatively, proteins known to interact with the C-terminus of A2A such as alpha-actinin, ARNO, USP4 and translin-associated protein-X could displace AC and therefore abrogate A2A-triggered cAMP-dependent signaling (84–88).

Our published and newer data clearly indicate that CD39 and changes in the nucleotide/nucleoside balance impact insulin-sensitivity, block mTOR activation (ATP-dependent) while boosting AMPK functions (adenosine-dependent process) (49). Although CD39 appears to be associated with enhanced T-cell survival, much as rapamycin and metformin have been shown to do, additional effects of CD39 include protection from P2X7-mediated apoptosis and the provision of nucleosides that activate A2A receptors, obviating AICD, promoting intracellular anabolism as well as purine salvage pathways.

Based on the effects of purinergic signaling on T cell memory and exhaustion, these findings suggest that CD39 targeting strategies might provide a new generation of checkpoint targeting modalities for the treatment of cancer and chronic infections (89, 90). In this context, CD39 inhibition is likely to be more effective than only targeting CD73 (91), because of its effects not only in arresting the generation of immunosuppressive adenosine, but also in promoting the accumulation of immunostimulatory eATP (92), as well as indirectly leading to the feed forward inhibition of CD73 (93).

Effects of eATP and CD39 on DCs

DCs control the activation of T cells *in vivo*, and their differentiation into specific functional lineages through the production of polarizing cytokines (62, 94). IL-1 β and IL-18, for example, promote the differentiation of Th17 and Th1 cells, respectively. In agreement with their important roles in shaping the immune response, the production of the biologically active forms of these cytokines is a highly regulated process. First, inactive proIL-1 β and proIL-18 are generated in response to TLR agonists or TNF α . A second stimulus such as uric acid crystals, amyloid- β or eATP is then needed to trigger the proteolytic activation of proIL-1 β and proIL-18 through a process controlled by the inflammasome (95–98).

The NLRP3 inflammasome catalyzes the production of active IL-1 β and IL-18 in response to a collection of structurally different signals (97). On such signal is eATP, which activates the NLRP3 inflammasome in DCs through a mechanism mediated by P2X7 (2, 99).

The importance of the NLRP3 inflammasome in the T-cell response is highlighted by the decrease in contact hypersensitivity (100) and Th1 and Th17 responses (101, 102) observed in NLRP3-deficient mice. Indeed, NLRP3 inflammasome activation is required for the development of autoimmune CNS inflammation (99, 103). Based on the activation of NLRP3 by eATP, CD39 is likely to modulate the production of polarizing cytokines by DCs and, indirectly, the T-cell response.

Initial support for a role of CD39 in the control of T-cell immunity by NLRP3 was provided by the observation of augmented contact hypersensitivity in CD39-deficient mice (104). More recent support for the role of CD39 expressed in DCs in the control of the NLRP3

inflammasome and T-cell responses was provided by our studies on tolerogenic DCs induced with IL-27 (99) (Fig. 4).

We reported that IL-27 signaling in DCs limits the differentiation of effector T cells and the development of EAE, while expanding FoxP3⁺ Tregs and Tr1 cells. In detailed transcriptional and epigenetic analyses we found that IL-27 promotes CD39 expression in DCs *in vivo* and *in vitro* through a mechanism mediated by STAT3. Further studies determined that CD39 in DCs limits the differentiation of Th1 and Th17 cells by depleting eATP, consequently reducing P2rX7-dependent NLRP3 activation and the production of IL-1 β and IL-18(99).

STAT3 deficiency restricted to DCs results in the spontaneous development of inflammation (105). These data suggest that other cytokines that activate STAT3 signaling and induce a tolerogenic phenotype in DCs such as IL-10 and IL-21 (106, 107) may trigger CD39-dependent regulatory pathways similar to those triggered by IL-27. In addition, STAT3 signaling is known to control the activity of microglia and macrophages; these cells also express CD39 (108–110). Thus, it is possible that the induction of CD39 expression constitutes a common immunoregulatory mechanism triggered by STAT3-activating cytokines in cells of the innate immune system.

Of note, eATP signaling through the P2Y₁₁ receptor in DCs inhibits LPS-induced IL-12p70 production while it increases the expression of anti-inflammatory IL-10 (111). Purinergic signaling in DCs also induce apoptosis, limiting immune responses (112). Thus, the net effects of CD39 on the regulation of DC function are likely dictated by multiple factors, including the balance in the P2X and P2Y expression by specific DC populations and local concentrations of eATP, ADP and other nucleotides.

Concluding remarks and future perspectives

The anti-inflammatory and modulatory effects of CD39 impact the T-cell response at multiple levels, promoting the differentiation, function and stability of effector and regulatory T cells. These effects result from the scavenging of eATP and the arrest of the modulation of T-cell activation and differentiation by P2R-dependent signaling. Hence, since eATP levels are high at sites of inflammation, CD39 expression provides a competitive advantage for T-cell dependent immunoregulation in the microenvironment of inflamed tissues.

Interestingly, IL-27 up-regulates CD39 expression in Tr1 cells and DCs through a mechanism mediated by STAT3 and AHR. It is likely that this anti-inflammatory transcriptional program is co-opted by other immune cells responsive to IL-27, such as FoxP3⁺ Tregs (113, 114) and effector T cells (60, 115, 116). Indeed, other STAT3-activating cytokines such as IL-10 may also promote CD39 expression. Therefore, CD39 expression and the modulation of purinergic signaling might provide a common mechanism of action for the anti-inflammatory effects of IL-27, IL-10 and other STAT3-activating cytokines.

Finally, the immunomodulatory effects of CD39 offer unique opportunities for therapeutic immunomodulation. The administration of exogenous CD39, either as a recombinant

protein, purified protein or in nanoparticles might provide a new approach to limit inflammation. This therapeutic approach is likely to be more efficient than existing strategies aimed at blocking P2X (69) or other receptors because of the ability not only to limit eATP signaling, but also to promote the generation of anti-inflammatory adenosine. Conversely, based on the expression of CD39 by exhausted T cells, the blockade of CD39 with small molecules or specific biological reagents such as monoclonal antibodies might provide a new approach to unleash protective immune responses in the context of chronic infections or tumor immunotherapy.

In summary, CD39 plays a significant role in the regulation of the T-cell response in the context of autoimmunity, infections and cancer. The further identification of the mechanisms by which CD39 controls the immune response will provide unique insights into the workings of the immune system in health and disease, and lead to the development of new and efficacious immunotherapeutic approaches.

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Trends box

- CD39 regulates T-cell activation, polarization and stability by scavenging immunostimulatory extracellular adenosine triphosphate (eATP) and promoting the generation of immunosuppressive adenosine.
- CD39 impacts T-cell response by acting directly on T cells, and also indirectly by modulating the activity of antigen presenting cells.
- The regulation of CD39 expression by cytokines and other immunological stimuli modulates the immune response in the context of inflammation, infections and cancer.
- CD39-targeted approaches offer new avenues for the therapeutic modulation of the immune response in autoimmunity, infections and cancer.

Figure 1. Modulation of the T-cell response by eATP

eATP boosts T-cell activation and modulates CD4⁺ T-cell differentiation. eATP-triggered activation of P2rX7 inhibits the differentiation of FoxP3⁺ Tregs and Tr1 cells, while it promotes the differentiation of Th17 cells. Moreover, eATP destabilizes FoxP3⁺ Tregs and induces apoptosis.

Figure 2. Effects of eATP on the polarization of CD4⁺ T cells

eATP (and hypoxia) activate HIF-1 α in T cells, leading to the ubiquitination and degradation of FoxP3 and AHR, and the suppression of FoxP3 Treg and Tr1 cell differentiation. Conversely, HIF-1 α stabilizes Rorc and the metabolic program of Th17 cells.

Figure 3. CD39 promotes the differentiation of Tr1 cells

During Tr1 cell differentiation, IL-27 triggered STAT3 activation promotes the expression of the transcription factors c-Maf and AHR, which transactivate the *I10* and *I21* promoters. AHR and HIF-1α associate with ARNT to control the expression of their target genes eATP activates HIF-1α signaling via P2rX7, displacing ARNT from its interaction with AHR, promoting AHR ubiquitination and degradation and effectively suppressing AHR-dependent signaling. CD39 limits eATP-induced HIF-1α signaling to promote Tr1 cell differentiation.

Figure 4. CD39 limits NLRP3 inflammasome activation in DCs

eATP activates the NLRP3 inflammasome in DCs via P2X7 signaling. IL-27 acts on DCs to induce CD39 expression through a STAT3-dependent mechanism. CD39 in DCs limits inflammation by limiting eATP-driven NLRP3 activation.

Table 1

eATP regulates the innate and adaptive immune response

	Cell type	P2 receptor	Functional response to eATP	Reference
Innate immune response	Eosinophils	P2Y2, P2Y6, P2X1, P2X7	Increases chemotaxis, Induce IL-8 production	Mu ller et al., 2010 Idzko et al., 2003
	Neutrophils	P2Y2	Improve migration, phagocytosis-mediated pathogen clearance Increases chemotaxis,	Chen et al., 2010;
	Monocytes/ Macrophages	P2Y2, P2X7	Activates NALP3-induced IL-1 β and IL-8 production Increases chemotaxis,	Elliott et al., 2009
	Dendritic cells	P2Y2, P2Y11, P2X7	Inhibits IL-12p70 and increases IL-10 production, activates NALP3-induced IL-1 β and IL-8 production	Idzko et al., 2002 Marteau et al., 2004 Mascanfroni et al., 2013
	naive CD4+ T cells	P2X7, P2X4	Amplifies TCR activation and IL-2 production, inhibits IL-27- mediated Tr1 differentiation reduce T-cell motility	Schenk et al., 2008, Yip et al., 2009, Mascanfroni et al., 2015; Wang et al., 2014
Adaptive immune response	FoxP3+ CD4+ T cells	P2X7	Induces apoptosis, inhibits FoxP3 expression, converts Treg cells in Th17	Schenk et al., 2011; Aswad et al., 2005
	CD8+ T cells	P2X7	Induces apoptosis	Heiss et al., 2008

Table 2

Ectonucleotidases expression on the immune cells

Cell type	Ectonucleotidase	Ligand	Function	Reference
Monocytes/ macrophages dendritic cells B cells	CD39	ATP, ADP	Self-limiting activation process	Lévesque et al., 2010
	CD39	ATP, ADP	Inhibits activation NALP3-mediated eATP	Mascanfroni et al., 2013
	CD39, CD73	ATP, ADP AMP	Class switch recombination	Schena et al., 2013
FoxP3+ CD4+ Treg cells	CD39, CD73	ATP, ADP AMP	Stabilization of FoxP3, prevent eATP- triggered apoptosis, adenosine mediated suppressive function	Sun et al., 2010; Schenk et al., 2011; Aswad et al., 2005
Tr1 cells	CD39	ATP, ADP	Promote Tr1 cell differentiation by eATP depletion, adenosine mediated suppressive function	Mascanfroni et al., 2015
Sup Th17 cells	CD39, CD73	ATP, ADP AMP	Adenosine mediated suppressive function	Chalmin et al., 2012