CD73 and CD39 ectonucleotidases in T cell differentiation: Beyond immunosuppression

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1. Introduction

Purinergic mediators, such ATP and adenosine, can be released into the extracellular space and act as positive or negative signals influencing the outcome of immune responses. Whereas intracellular ATP is primarily used to drive energy-requiring processes, such as active transport, cell motility and biosynthesis, extracellular ATP is considered a powerful signaling molecule [1]. ATP that is released from damaged or stressed cells acts as a “find-me” signal that guides phagocytes to inflammatory sites, promoting the clearance of damaged cells [2]. ATP also induces pro-inflammatory responses, such as inflammasome activation and the subsequent release of inflammatory cytokines [3]. In contrast, extracellular adenosine accumulation serves as “reporter” of excessive tissue damage. It has been shown that adenosine acts on diverse immune cells, mediating mainly anti-inflammatory effects by inhibiting activated immune cells in a negative feedback loop to prevent additional tissue damage [4].

In healthy tissues, ATP is almost exclusively present inside the cells, reaching millimolar concentrations, whereas extracellular ATP concentration is in the low nanomolar concentrations [5]. A study using a bioassay approach to measure ATP concentrations in several tissues, has demonstrated that ATP is in the hundreds micromolar range in the tumor interstitium [6]. In addition to its release during tissue damage, ATP may be released by immune cells upon activation. It has been reported that T cells and neutrophils release ATP through pannexin 1 channels [7,8] whereas B cells release ATP stored in calcium sensitive vesicles. Once in the extracellular environment, ATP is hydrolyzed by plasma membrane nucleotidases to generate ADP, AMP and finally adenosine [9]. Evidence of adenosine bioavailability has been demonstrated by the fact that A2A receptors are readily activated in CD8+ and CD4+ T cells, since A2A receptor deficiency on T cells affects their numbers in the periphery [10] and effector-memory differentiation in all T cells [11]. This demonstrates that at least the extracellular levels of adenosine detected by T cells under homeostatic conditions are enough to signal through A2A receptors.

The extracellular concentration of adenosine depends on: (i) the balance between its release from cells, (ii) its re-uptake by the bi-directional adenosine transport processes, and (iii) its
generation by ectonucleotidases on the cell surface [12]. CD39 (NTPDase-1) and CD73 (ecto-5′-nucleotidase) are two cell-surface ecto-enzymes that dephosphorylate ATP into its metabolites, ADP, AMP and adenosine, in a tightly regulated process [13]. The conversion of ATP into AMP is catalyzed by CD39, [14], whereas CD73, a glycosyl phosphatidylinositol (GPI)-linked membrane-bound glycoprotein, catalyzes the dephosphorylation of AMP into adenosine [15]. Whereas the conversion of ATP to AMP by CD39 can be reversed by the actions of the extracellular diphosphate kinases NDP kinase and adenylyl kinase, the conversion of AMP into adenosine by CD73 is reversible only following intracellular transport of adenosine, where it can be converted to AMP by adenosine kinase [16].

CD39 and CD73 have been widely considered pivotal in the generation of immunosuppressive microenvironments through adenosine production [13,17]. In accordance with this idea, CD39 and CD73 ectonucleotidases are reported as active players in several diseases, such as cancer, autoimmunity, allergy, and ischemia-reperfusion injury (reviewed in [17]). In this review, we explore recent evidence showing that, besides its immunosuppressive role, CD39 and CD73 ectonucleotidases may also be involved in the regulation of other aspects of T-cell biology, such as naive cell homeostatic survival, memory cell survival and differentiation.

2. CD39 and CD73 expression in immune cells

CD39 and CD73 are expressed at different levels in a variety of tissues, including the heart, placenta, lung, liver, colon, brain and kidney [18–20]. Additionally, these ectonucleotidases are highly upregulated in a large number of tumors, such as melanoma, leukemia, pancreatic, liver, gastric, colon and breast cancer [13,21]. Interestingly, CD39 and CD73 ectonucleotidases are also expressed by immune cells, including monocytes, neutrophils, dendritic cells, myeloid-derived suppressor cells, B lymphocytes, and some T-cell subsets [22–27].

2.1. T-cell subsets expressing CD39 and CD73 ectonucleotidases

CD39 and CD73 ectonucleotidases are highly expressed in murine CD4+ Foxp3+ regulatory T cells (Treg cells) and have been extensively used as markers of this T-cell subpopulation [23,28]. Human Treg cells have been reported to express CD39 on their surface, but in contrast to murine Treg cells, CD73 is predominantly intracellularly expressed. It has been demonstrated that CD39 and CD73 ectonucleotidases are key in adenosine production by Treg cells and therefore are considered to be part of their immunosuppressive arsenal [23].

Some reports have shown that in addition to Treg cells, other T-cell subsets express the CD39 and CD73 ectonucleotidases. Thpp cells are primed CD4+ T cells that remain as proliferating but uncommitted precursor cells. These cells express high levels of CD44, secrete IL-2 and can subsequently differentiate into Th1 and Th2 cells in appropriate cytokine environments [29]. It has been described that these cells express CD73 [30] and are able to produce adenosine and suppress the proliferation of CD4+ or CD8+ T cells in in the presence of exogenous AMP [28].

Ghiringhelli and collaborators have demonstrated that Th17 cells generated with TGF-β and IL-6 express both ectonucleotidases. Moreover, they suggest that Th17 cells are able to produce adenosine and limit IFN-γ and granzyme B production by CD4+ and CD8+ T cells, thus exhibiting protumoral activity [31]. However, these results must be interpreted with some caution, since the reduction in IFN-γ production by CD4+ T cells was observed only at high Th17:Teff ratios (1:1 ratio). Moreover, several studies have demonstrated that Th17 cells present antitumor activity [32–37] even when ectonucleotidase expression by Th17 cells has been demonstrated [38]. Interestingly, we have observed that other T helper subsets, such as Th1 cells, also express both ectonucleotidases in vivo (unpublished data), suggesting that in addition to Treg cells, other T helper subsets may also be able to produce adenosine. Thus, it is tempting to speculate that CD39 and CD73 ectonucleotidases in T helper subsets may be required for other processes in addition to promoting immunosuppressive microenvironments through adenosine production.

Two reports have shown that a subset of human and murine memory CD4+ T cells express CD39 [39,40]. Although these CD39+ memory T cells have ATPase activity, they do not possess immunosuppressive activity. In consequence, it has been suggested that CD39 expression by memory T cells may instead contribute to dampening ongoing inflammatory processes and/or rescue these cells from ATP-induced apoptosis/necrosis [40]. In agreement with previous evidence from the literature [15,41], we found that naïve CD8+ T cells express CD73 and downregulate its expression upon activation (unpublished results). Although currently there is no evidence of a role of CD73 in CD8+ T-cell differentiation, these results suggest that CD73 downregulation may be necessary to prevent autocrine adenosine signaling during T-cell activation, thus allowing the transition to effector cells (Fig. 1).
2.2. Other immune cells expressing CD39 and CD73 ectonucleotidases

In addition to T cells, evidence from the literature demonstrates that neutrophils and B cells also express CD39 and/or CD73 ectonucleotidases. It has been reported that extracellular ATP and adenosine both play critical roles in the polarization and migration of neutrophils via tightly coordinated processes that involve the activation of P2Y2 and A3 receptors [7]. The group of Junger has further demonstrated that CD39 localizes at the leading edge where most of the ATP released by neutrophils accumulates. This suggests a dual role for CD39 in the rapid decrease of ATP concentrations at the leading edge and in the initiation of adenosine formation, which contributes to directed migration of neutrophils [42]. Moreover, a recent report has shown that ATP is stored in secretory vesicles in human B cells and that may be released upon B cell receptor stimulation. This ATP may be hydrolyzed to adenosine by the tandem action of CD39 and CD73 ectonucleotidases expressed by B cells, inducing an autocrine adenosine signaling which is necessary for class switch recombination. In agreement with this evidence, patients with impaired class switching antibody responses present B cells lacking CD73 expression [43]. All these data provide evidence of the involvement of CD39 and CD73 ectonucleotidases in adenosine production and in initiating an autocrine adenosine signaling in immune cells.

2.3. Factors that induce CD39 and CD73 expression

It has been described that ectonucleotidase expression is regulated by several transcription factors, including Sp1 [44], Stat3, and Gfi-1 [31]. CD39 and CD73 expression and function are upregulated under hypoxic conditions [45] as well by the presence of TGF-β1 and IL-6 [31,46], type I IFNs [47] and Wnt signaling pathway agonists [48]. In contrast, inflammatory cytokines, such as IL-4, IL-12, IL-21 and IFN-γ, can prevent CD73 expression induced by TGF-β1 [46].

3. Adenosine and immunosuppression

Adenosine has been mainly related to immunosuppression during the anti-tumor immune response. In this section, we gather all of the evidence demonstrating a role of this nucleoside in suppressing T-cell responses and promoting tumor immune evasion.

3.1. Adenosine as an immunosuppressive molecule for T cells

Adenosine signaling is mediated by four G-protein-coupled adenosine receptors: A1, A2A, A2B and A3. The A1 and A3 adenosine receptors are coupled to the G1/o subunit and their stimulation leads to the inhibition of adenyl cyclases, decreasing intracellular cyclic AMP (cAMP) levels. On the other hand, A2A and A2B adenosine receptors are coupled to the Gs subunit, which stimulates cAMP production and PKA activation [49,50]. Stimulation of A2A and A2B receptors by adenosine has been reported to mediate suppressive effects on T cells, dendritic cells, neutrophils, macrophages and other immune cells [51]. The A2A receptor is the predominant subtype found on T cells and is induced upon T-cell activation [17].

A2A receptor stimulation and the subsequent activation of PKA lead to cAMP responsive element-binding protein (CREB) phosphorylation, which can bind to the p65 subunit of the NF-κB transcription complex inhibiting NF-κB-driven gene expression [52]. Additionally, there is evidence that indicates that PKA directly inhibits the Ras/MAP kinase pathway [53], which is involved in T-cell proliferation [54]. Accordingly, it has been described that extracellular adenosine prevents the activation, proliferation and expansion of T cells [23,55] and may also inhibit cytotoxicity and cytokine production [56]. Moreover, A2A receptor stimulation inhibits Th1 and Th2 cell differentiation by decreasing naive T-cell proliferation and IL-2 production [57]. Stimulation of the A2A receptor has also been shown to promote the generation of Foxp3+ and LAG-3+ Treg cells and reduce Th17 cell differentiation by increasing TGF-β and decreasing IL-6 production by splenocytes [58].

3.2. Adenosine production by regulatory T cells

Treg cells are responsible for inducing and maintaining peripheral tolerance [59]. One of the suppressive mechanisms attributed to Treg cells is metabolic disruption, ascribed in part to the expression of CD39 and CD73 ectonucleotidases and the concomitant adenosine production [23,28,60]. The contribution of CD39 in immunoregulation by Treg cells is supported by the occurrence of alopecia areata and experimental autoimmune encephalomyelitis in CD39-deficient mice and by the failure of CD39−/− Treg cells to suppress contact hypersensitivity [61]. The inhibitory effect of adenosine on effector T cells is mediated by the blockade of T-cell receptor signaling due to an increase in cAMP after A2A receptor stimulation [62,63]. In addition to its role in the generation of adenosine and the creation of an immunosuppressive microenvironment, CD39 expression has also been related to the ability of Treg cells to survive in ATP-rich sites, such as in inflamed tissue [60].

Interestingly, Sitkovski and collaborators recently presented evidence of a positive autocrine feedback loop via A2A adenosine receptors on Treg cells. This group demonstrated that T-cell activation in the presence of an A2AR agonist resulted in the expansion of Treg cells and the generation of Treg cells with stronger immunoregulatory activity [64].

3.3. Adenosine as a mechanism of immune escape in cancer

It has been reported that there is an important increase in CD39 and CD73 expression in blood neoplasias, such as leukemia and lymphoma, as well as in several solid tumors [21]. Thus, adenosine generation in the tumor microenvironment has been proposed to be one of the mechanisms used by tumors to create an immunosuppressive microenvironment and avoid tumor destruction. Adenosine-related tumor immune escape is mediated by several mechanisms: (i) the inhibition of activation, expansion and cytokine production by T cells [56,65,66], (ii) the impairment of T-cell and NK-cell cytotoxic activity [56,67], (iii) the induction of Treg cells and MDSCs [26,58], (iv) the inhibition of the maturation and activation of dendritic cells [68], and (v) the promotion of M2 conversion in tumor-associated macrophages [69], among many others.

In addition to its effect on immune populations, it has been reported that adenosine may also regulate intrinsic functions of tumor cells, such as proliferation, apoptosis, angiogenesis and metastasis (reviewed in [70]).

4. Other ectonucleotidase functions

In addition to their role in adenosine production, CD39 and CD73 are involved in other processes, such as ATP clearance, co-stimulation and cell adhesion. These aspects will be discussed in the following section.

4.1. CD39 and ATP clearance

Extracellular ATP can be sensed by purinergic P2 receptors. These receptors include cation-selective receptor channels (P2X)
and metabotropic G protein-coupled receptors (P2Y). Purinergic P2 receptors are expressed in immune and epithelial cells where ATP sensing induces mainly pro-inflammatory responses [71]. It has been demonstrated that high doses of ATP induce necrotic lysis through P2X7 receptor signaling in T cells [72]. Thus, cells provided with a mechanism to deplete ATP, such as CD39-mediated hydrolysis of ATP, may be more fit compared with cells lacking CD39 expression when entering ATP-rich sites.

The first evidence in support of this hypothesis came from the observation that CD39 expression on Treg cells is required not only to exert their regulatory function but also to proliferate. Regulatory T cells are highly sensitive to ATP-induced necrotic lysis through purinergic receptor P2X7 signaling [72]. Importantly, inhibition of CD39 catalytic activity has been shown to reduce the proliferative capacity of Treg cells in the presence of extracellular ATP [60]. Thus, it has been proposed that CD39 expression by Treg cells not only mediates immunosuppression through the production of adenosine but is also required to lower extracellular ATP concentration below the toxic threshold [60].

We and others [46] have demonstrated that CD39 expression is upregulated in activated CD4+ T cells. Moreover, we observed that, in addition to Treg cells, other effector T helper subsets, such as Th1 and Th17 cells, also express CD39, and its expression is upregulated following the infiltration of ATP-rich sites, such as the tumor microenvironment (unpublished results).

In line with all the evidence pointing to a role of CD39 ectonucleotidase in depleting ATP, our unpublished data shows that in vitro-generated Th17 cells that express higher levels of CD39 expression are less susceptible than CD39− Th17 cells to ATP-induced necrotic lysis. Moreover, when transferred into tumor-bearing mice, CD39- Th17 cells rapidly convert to CD39+ cells within the tumor microenvironment. Although all of this evidence does not rule out a role of CD39 in the generation of adenosine and immunosuppressive microenvironments, it suggests that this ectonucleotidase is upregulated in effector T-cell populations and provides a mechanism of survival in ATP-rich sites.

4.2. CD73 as a co-stimulatory molecule

It has been proposed that, besides its enzymatic activity, CD73 may function as a co-stimulatory molecule for T cells. Cross-linking CD73 with monoclonal antibodies (mAb) in the presence of PMA or in combination with submithogenic concentration of anti-CD3 mAb stimulates human peripheral blood T cells to proliferate, express CD25 and secrete IL-2 [73,74], suggesting that CD73 could trigger an activation signal similar to other GPI-anchored lymphocytes antigens, such as Thy-1 and Ly-6 [75]. Other studies revealed that naive CD45RA+ CD45RO+ CD8+ T cells, which express CD73, have low responsiveness to activation through immobilized anti-CD3, which can be overcome when CD3 and CD73 are simultaneously cross-linked by immobilized anti-CD73 and anti-CD3 mAb [76]. This suggests that CD73 could reduce the threshold for activation of naive T cells when they first encounter antigen [77].

4.3. CD73 in cell adhesion

The addition of anti-CD73 mAb to in vitro cultured endothelial cells can inhibit the binding of lymphocytes, suggesting that CD73 expression by endothelial cells may mediate the binding of lymphocytes to the endothelium [78]. In the same line, CD73 expression on follicular dendritic cells mediates the adhesion between B cells and follicular dendritic cells in vitro, which can be inhibited by the presence of an anti-CD73 mAb [22]. On T lymphocytes, the engagement of CD73 by an anti-CD73 mAb increases their binding to cultured endothelial cells in an LFA-1-dependent fashion, suggesting that CD73 may control the activation step in the multistep cascade of lymphocyte extravasation [79].

5. CD73 and adenosine in the control of T-cell differentiation

Upon activation, naive T cells give rise to a heterogeneous progeny of short-lived effector cells (SLEC) and memory precursor cells (MPEC) that provide acute and long-term protection respectively. The formation of memory T cells is an important feature of adaptive immunity and rely at least in part on the ability of T cells to survive and/or self-renew in an antigen-independent manner [80]. In the following section, we will discuss recent evidence regarding the role of CD73 and adenosine in the maintenance of naïve and memory T cell phenotype, including naïve T cell homeostatic survival, memory T cell survival, and stemness of memory-like T cells. We will discuss recent studies supporting the idea that CD73-mediated adenosine signaling can restrain T cell effector differentiation favoring the formation of long-lived memory T cells.

5.1. Akt inhibition and arrest of CD8 T-cell differentiation

It has been proposed that the magnitude of TCR, costimulation and cytokine signaling are responsible of fine-tuning CD8+ T cell fate. While strong T cell stimulation promotes the differentiation of short-lived effector T cells (SLEC), weak T cell stimulation induces a memory phenotype which is characterized by long-term survival capacity [81]. Importantly, several inputs that control this fate decision converge at the activation of Akt pathway [82]. Accordingly, recent studies have highlighted the role of the Akt pathway as a central node in memory versus effector differentiation of CD8+ T cells (reviewed in [82]). Thus, Akt signaling has been shown to control several transcriptional regulators of T cell differentiation such as FOXO, mTOR and the Wnt/β-catenin axis (Fig. 2). Interestingly, A2A receptor stimulation decreases TCR-mediated activation of the Akt pathway, supporting a role of extracellular adenosine in modulating T cell activation signaling [10,83].

All of this evidence sustains the hypothesis that adenosine signaling through A2A receptors may restrain T-cell differentiation by reducing Akt activation (Fig. 2). In agreement with this idea, Akt inhibition has shown to enhance the formation of long-lived memory CD8+ T cells [84,85]. Furthermore, Akt pharmacological inhibition during ex-vivo expansion of tumor infiltrating CD8+ T cells promotes a phenotypic (CD62L+), transcriptional (Tcf7, Lef1, Foxo, Klf2, and Sell) and metabolic profile (increased fatty acid oxidation) characteristic of long-lived memory CD8+ T cells. All of these features were associated with enhanced T-cell persistence upon adoptive transfer and tumor regression following adoptive T-cell therapy [84]. Conversely, it has been demonstrated that sustained Akt activation enhances effector functions, drives differentiation into terminal effector cells and reduces the potential of CD8+ T cells to survive and differentiate into memory cells [86,87].

5.2. Inhibition of CD127 down-regulation and memory T-cell survival

In contrast to all of the evidence pointing to a role of adenosine in generating immunosuppressive microenvironments that favor tumor progression [85,66]. Linden and collaborators demonstrated that global deletion of A2A receptors impairs memory CD8+ T-cell accumulation in tumors and promotes tumor progression. These results were recapitulated in Adora2a−/− Lck-Cre+ mice, which lack A2A receptors, specifically in T cells [88]. Additionally, deficiency in the A2A receptor in CD8 T cells led to impaired survival upon adoptive transfer into tumor-bearing mice, which correlated
with reduced IL-7Rα (CD127) expression [11]. These data demonstrate a T-cell intrinsic mechanism involving an A2A receptor-dependent control of CD127 expression in the survival of long-lived memory CD8+ T cells.

Remarkably, the above-mentioned results demonstrate that adenosine signaling is active in memory CD8+ T cells and functions to maintain CD127 expression and T-cell survival. However, it is not clear yet how adenosine availability is controlled in these scenarios. CD8+ T cells express CD39 and CD73 ectonucleotidases [39,89,90], supporting the hypothesis that adenosine may be generated by T cells themselves through CD39 and CD73-mediated extracellular ATP hydrolysis. Thus, ectonucleotidase expression by T cells may support an autocrine adenosine signaling that is necessary for CD127 expression and survival of memory CD8+ T cells. In support of this idea, we have shown that memory and naive CD8+ T cells, but not effector T cells, express high levels of CD73 which is responsible for generating extracellular adenosine (unpublished data, Fig. 1). Moreover, tumor infiltrating CD8+ T cells that lack CD73 expression, express markers of SLECs, such as KLRG1, and downregulate markers that are associated with long-lived memory T cells, such as CD127 and CD62L (unpublished data). Because CD73 is able to generate adenosine by the hydrolysis of AMP, these data suggest that CD73-driven adenosine production mediates an autocrine loop that arrests T-cell differentiation, thus favoring the maintenance of long-lived memory T cells.

5.3. Inhibition of CD127 down-regulation and homeostatic naive T-cell survival

New evidence supports the hypothesis that adenosine may regulate CD8+ and CD4+ naive T-cell homeostatic survival and peripheral maintenance. A recent report has demonstrated that adenosine favors naive CD8+ and CD4+ T-cell homeostatic survival by preventing CD127 downregulation following T-cell receptor stimulation by low affinity self-peptide-MHC complexes. This effect is mediated by adenosine-induced activation of PKA, followed by the reduction of the TCR-mediated activation of the Akt pathway. The downregulation of CD127 correlates with less persistence in vivo, due to decreased anti-apoptotic Bcl-2 expression, which is a downstream target of IL-7 signaling [10].

6. Concluding remarks

Extracellular ATP and adenosine are considered to be important factors in regulating immune responses. Whereas ATP acts as a danger signal that promotes inflammation, extracellular adenosine acts as an immunoregulatory signal that modulates adaptive and innate immune response functions. As a consequence, the ATP/adenosine balance is crucial in immune homeostasis. CD39 and CD73 are two ectonucleotidases that cooperate in the generation of extracellular adenosine through ATP hydrolysis. These ectonucleotidases may be considered to be “immunological switches”, tiltng the balance from inflammation to immunosuppression. Although the role of adenosine in inducing immunosuppression is well established, recent evidence has shown that this nucleoside may also affect other T-cell biology processes, such as T-cell homeostasis, memory survival and differentiation. CD39 and CD73 expression is finely regulated during the transition of naive/memory T cells to effector cells. Thus, through ectonucleotidase expression, naive/memory T cells are endowed with the ability to produce adenosine and lose this ability as they transition to more differentiated cells. We propose that ectonucleotidases that are expressed by naive/memory T cells allow adenosine production, which signals in an autocrine manner, resulting in the arrest of T-cell differentiation in the absence of strong TCR signaling.

Fig. 2. The Akt pathway as a central node in CD8+ T-cell memory versus effector differentiation. Signals through the TCR, co-stimulatory molecules and cytokine receptors converge to activate the Akt pathway. The magnitude of Akt activation fine-tunes T-cell differentiation. Akt can inhibit the activity of memory-promoting transcription factors, such as FOXO and TCF/LEF/β-catenin. On the other hand, Akt activates mTORC1, which in turn promotes T-cell differentiation by facilitating anabolic metabolism and promoting the expression of T-bet. Thus, Akt activation drives CD8+ T cells to terminal differentiation at the expense of memory formation. A2A receptor stimulation drives cAMP accumulation followed by PKA activation. PKA is a negative regulator of Akt, thus extracellular adenosine may regulate T cell differentiation by preventing Akt activation.
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References


