

References

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Response:

TCD response to hydroxyurea therapy

We thank the authors for summarizing the Belgian experience with transcranial doppler (TCD) for children with sickle cell disease (SCD). Although their data are retrospective, they describe significant differences in the TCD velocities of children treated with hydroxyurea compared with those who were not. Similar to our findings,¹ children with highest baseline TCD velocities had the greatest treatment-related decreases. Their data also provide additional support for the use of hydroxyurea for primary and even secondary stroke prevention in children with SCD. We are encouraged that these pilot data further document the benefits of hydroxyurea for children with SCD, yet we maintain that treatment at the maximum tolerated dose is preferred.² As we concluded in our paper, controlled multicenter prospective trials are needed to determine the efficacy of hydroxyurea therapy in these clinical settings.

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To the editor:

Hydrolysis of extracellular ATP and immune suppression: humans versus mice

We read with interest the recent article by Borsellino and colleagues on the role of CD39 and CD73 for the suppressor activity of Foxp3⁺ regulatory T cells (T regs).¹

Because the major sources of extracellular ATP are injured cells leaking cytoplasmic content, degranulating platelets, and endothelial cells under shear stress, extracellular ATP may represent a constitutive endogenous molecule that signals tissue stress and injury. Borsellino and colleagues proposed that the immune suppressive activity of T regs is due, at least in part, to their capacity to remove ATP from the extracellular space through the enzymatic activity of CD39 and CD73 expressed on their membrane.

It has been recently shown that T regs exert immune suppression by elevating intracellular cyclic AMP (cAMP) concentration in target cells.² As suggested by Borsellino and others, T regs might do so by degrading ATP to adenosine. In turn, adenosine activates adenylyl cyclases by triggering the cognate Gs-protein coupled receptor A2a.^{1,3} However we would like to point out that in human cells ATP can act as a direct cAMP-elevating agent thus delivering a potent anti-inflammatory signal. This is because human, but not murine, cells express the purinergic receptor P2Y₁₁ that is the only P2 purinergic receptor coupled to adenylyl cyclases activation.⁴

Cells expressing P2Y₁₁ include dendritic cells (DCs), macrophages, T lymphocytes, and natural killer cells. Human DCs-exposed to micromolar concentrations of extracellular ATP do not undergo "classic" maturation. In fact, although ATP-stimulated

DCs up-regulate costimulatory membrane molecules, they also display blocked pro-inflammatory cytokine and chemokine production including tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-12, CCL2, CCL3, CCL5, and CXCL10, while IL-10 and IL1Ra are either unaffected or up-regulated.⁵⁻⁷ Moreover, extracellular ATP induces DCs to produce large amounts of Thrombospondin-1 and synergizes with interferon- γ (IFN- γ) in up-regulating indoleamine 2,3 dioxygenase, turning DCs into tolerogenic antigen presenting cells.⁸ These effects are mimicked by the nonhydrolyzable ATP analog ATP- γ -S and by several cyclic AMP elevating agents, as well as by the administration of cell permeable cAMP analogs.^{8,9} Moreover, extracellular ATP has proven able to inhibit T lymphocyte and NK cell proliferation and cytokine production as well as NK-mediated cytotoxicity, associated with increased intracellular cAMP concentration.¹⁰

Extracellular ATP released from injured cells might not act as an activating danger signal but it might rather represent a negative feedback for immune cells to limit self-harmful excessive inflammation in the context of extensively damaged human tissues. This view suggests that extracellular ATP hydrolysis operated by human CD39⁺ T regs might have more complex physiologic consequences than blunt immune suppression.

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Response:

Hydrolysis of extracellular ATP by CD39⁺ Treg cells: context matters!

We thank our colleagues for their comments as they stress the complex role extracellular adenosine triphosphate (ATP) actually plays in immune regulation. This applies even more so as there are indeed apparent differences between mice and humans that may result in species-specific signaling pathways. These differences comprise not only the differential expression of P2Y11 receptors but also the cellular distribution of CD39. In the mouse, the ectoenzyme is expressed constitutively on all Foxp3⁺ T regulatory (Treg) cells, whereas in humans the expression is restricted only to a specific Treg subset.¹ While these differences may complicate the transfer of experimental results between species, for the immune regulation by CD39⁺ Treg cells we still regard ATP primarily as a 'classical' danger signal for both humans and mice.

In principle, we agree that the role of extracellular ATP hydrolysis is likely to be more complex than just 'blunt immune suppression.' Nothing is black or white; everything depends on the context. A prominent example is mouse transforming growth factor (TGF)- β , which normally drives the generation of immune-suppressive Treg cells but in the presence of IL-6 converts into a differentiation factor for pro-inflammatory Th17 cells.² The same also applies for ATP. As quoted already in the report by Borsellino et al,¹ exposure of immature human dendritic cells (DCs) to ATP alone triggers only an incomplete maturation and produces cells unable to secrete pro-inflammatory cytokines. The cells are in fact tolerogenic as they release thrombospondin-1 and express indoleamine 2,3 dioxygenase.³ The same study,³ however, also revealed that ATP triggers the up-regulation of IL-23. This is in line with Schnurr et al,⁴ who showed that also in the presence of bacterial stimuli ATP potentiates the induction of IL-23, while blocking the generation of IL-12. Notably, IL-23 is the key-survival factor for Th17 cells, a recently discovered subset of effector/memory cells involved in autoaggressive immune attacks.² Thus, depending on the environment, ATP acts on human immature DCs not only as an enhancer of tolerance-induction. During infection and ongoing immune reactions it may instead promote the expansion of pro-inflammatory Th17 cells.

Extracellular ATP plays a similar role also in the regulation of innate immunity. The generation of pro-IL-1 β is induced by TLR-ligands such as LPS or CpG. Some reports indicate that ATP is involved in the release of the cytokine as it triggers the shedding of IL-1 β -filled microvesicles from activated monocytes or mature DC.^{5,6} More importantly, other studies have shown that extracellular ATP actually regulates the inflammasome-dependent conversion

of pro-IL-1 β into the mature cytokine.⁷ Thus, in the presence of TLR ligands, ATP acts as an immediate inducer of pro-inflammatory cytokines. On human Treg cells, CD39 is expressed on a subset of effector/memory-like cells (T_{REM}).¹ T_{REM} cells accumulate inside the inflamed tissue⁸ to which they have been attracted by factors such as the CCR6-ligand CCL20. The chemokine is released by activated leukocytes during infection and inflammation, so that they should encounter extracellular ATP usually only in a context where it acts as a pro-inflammatory danger signal.

In the absence of other signals indicating inflammation or infection, extracellular ATP may indeed exhibit some suppressive effects. Although the suggested inhibitory role of the P2Y11 receptor still needs to be established, it could directly inactivate human T cells by raising intracellular cAMP levels. However, high concentrations of ATP are toxic also for mouse T cells, where the effect is mediated by P2 \times 7 receptors. Mouse Treg cells are even particularly sensitive to the nucleotide⁹ and it is namely the ecto-ATPase CD39 that allows them to cope with the elevated ATP levels in inflamed sites.¹ The close linkage of the ATPase-activity of CD39 to the activation status of the Treg cell in mice clearly points to an immune-suppressive function. Restricted expression of the enzyme by a subset of Treg cells acting inside inflamed tissue suggests that also in humans its primary function is the removal of an activating danger signal.¹ The generation of adenosine by CD39 and CD73, as demonstrated by Deaglio et al,¹⁰ may further add to the anti-inflammatory effect.

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