Correspondence: Andrea la Sala, Laboratory of Molecular and Cellular Immunology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) San Raffaele, Via dei Bonacolsi, 81, 00163 Rome, Italy; e-mail: andrea.lasala@sanraffaele.it.

References

1. Borsellino G, Kleinewietfeld M, Di Mitri D, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood. 2007;110:1225-1232.
2. Bopp T, Becker C, Klein M, et al. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. J Exp Med. 2007;204:1303-1310.
3. Deaglio S, Dwyer KM, Gao W, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med. 2007;204:1257-1265.
4. Abbracchio MP, Burnstock G, Boeynaems JM, et al. International Union of Pharmacology LVIII: Update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Pharmacol Rev. 2006;58:281-341.
5. la Sala A, Ferrari D, Corinti S, et al. Extracellular ATP induces a distorted maturation of dendritic cells and inhibits their capacity to initiate Th1 responses. J Immunol. 2001;166:1611-1617.
6. la Sala A, Sebastiani S, Ferrari D, et al. Dendritic cells exposed to extracellular adenosine triphosphate acquire the migratory properties of mature cells and show a reduced capacity to attract type 1 T lymphocytes. Blood. 2002;99:1715-1722.
7. Horckmans M, Marcet B, Marteau F, et al. Extracellular adenine nucleotides inhibit the release of major monocyte recruiters by human monocyte-derived dendritic cells. FEBS Lett. 2006;580:747-754.
8. Marteau F, Gonzalez NS, Communi D, et al. Thrombospondin-1 and indoleamine 2,3-dioxygenase are major targets of extracellular ATP in human dendritic cells. Blood. 2005;106:3860-3866.
9. Ferrari D, Gorini S, Callegari G, la Sala A. Shaping immune responses through the activation of dendritic cells' P2 receptors. Purinergic Signal. 2007;3:99-107.
10. Miller JS, Cervenka T, Lund J, Okazaki IJ, Moss J. Purine metabolites suppress proliferation of human NK cells through a lineage-specific purine receptor. J Immunol. 1999;162:7376-7382.

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-1β 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-1β-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-1β 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 tissue[8] 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 P2×7 receptors. Mouse Treg cells are even particularly sensitive to the nucleotide[9] 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.

Markus Kleinewietfeld, Diletta DiMitri, Alexander Sternjak, Adamo Diamantini, Giovanna Borsellino, Luca Battistini, Olaf Rötzschke, and Kirsten Falk

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Kirsten Falk or Olaf Rötzschke, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str. 10, D-13125 Berlin, Germany; e-mail: falk@mdc-berlin.de or roetzsch@mdc-berlin.de.

References

1. Borsellino G, Kleinewietfeld M, Di Mitri D, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood. 2007;110:1225-1232.
2. Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and

the expanding diversity of effector T cell lineages. Annu Rev Immunol. 2007;25: 821-852.

3. Marteau F, Gonzalez NS, Communi D, Goldman M, Boeynaems JM. Thrombospondin-1 and indoleamine 2,3dioxygenase are major targets of extracellular ATP in human dendritic cells. Blood. 2005;106:3860-3866.
4. Schnurr M, Toy T, Shin A, Wagner M, Cebon J, Maraskovsky E. Extracellular nucleotide signaling by P2 receptors inhibits IL-12 and enhances IL-23 expression in human dendritic cells: a novel role for the cAMP pathway. Blood. 2005; 105:1582-1589.
5. MacKenzie A, Wilson HL, Kiss-Toth E, Dower SK, North RA, Surprenant A. Rapid secretion of interleukin-1beta by microvesicle shedding. Immunity. 2001; 15:825-835.
6. Ferrari D, La Sala A, Chiozzi P, et al. The P2 purinergic receptors of human dendritic cells: identification and coupling to cytokine release. Faseb J. 2000; 14:2466-2476.
7. Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nat Rev Immunol. 2007;7:31-40.
8. Kleinewietfeld M, Puentes F, Borsellino G, Battistini L, Rotzschke O, Falk K. CCR6 expression defines regulatory effector/memory-like cells within the CD25(+)CD4+ T-cell subset. Blood. 2005;105:2877-2886.
9. Aswad F, Kawamura H, Dennert G. High sensitivity of CD4+CD25+ regulatory T cells to extracellular metabolites nicotinamide adenine dinucleotide and ATP: a role for P2X7 receptors. J Immunol. 2005;175:3075-3083.
10. Deaglio S, Dwyer KM, Gao W, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med. 2007;204:1257-1265.

To the editor:

Genotype-phenotype correlation in cases of juvenile myelomonocytic leukemia with clonal *RAS* mutations

In a recent issue of *Blood*, Matsuda et al reported 11 children with juvenile myelomonocytic leukemia (JMML) and clonal *NRAS* or *KRAS* mutations.[1] Three patients showed improvement of various clinical and laboratory features over a 2- to 4-year period without chemotherapy or hematopoietic stem cell transplantation (HSCT). The authors correlate the comparatively mild course with a specific mutation predicting a glycine-to-serine substitution at position 12 in the NRAS or KRAS protein (G12S), and suggest that "no chemotherapy may be a recommended management" for JMML patients with NRAS/KRAS^{G12S}.

We have some reason to believe that these conclusions are premature. Available data do not support that RAS^{G12S} has weaker oncogenic activity than substitutions with valine, arginine, or aspartic acid. Interestingly, the authors show that myeloid progenitor cells from their patients with G12S respond to granulocyte macrophage–colony stimulating factor (GM-CSF) in a comparable manner as other mutants (Figure 1B in Matsuda et al[1]). Others reported that HRAS^{G12S} led to focus induction in NIH3T3 cultures with a similar potency as substitutions with arginine or aspartic acid.[2]

We argue that the clinical course observed in the 3 children is not uncommon in JMML cases with similar hematologic features and age. The European Working Group of Myelodysplastic Syndromes in Childhood (EWOG-MDS) has previously shown that platelet count 33×10^9/L or more and hemoglobin F less than 15% at diagnosis identifies a prognostically favorable subgroup in JMML with a 40% to 70% probability of survival at 2 to 4 years without HSCT.[3] The relatively favorable course in the Matsuda patients is also because all 3 were less than 1 year old at diagnosis.[4] It is known that infants with JMML without severe thrombocytopenia at diagnosis may experience transient improvement even without treatment.[5]

To examine whether RAS^{G12S} is overrepresented in JMML patients with less aggressive disease (defined as survival ≥ 3 years without HSCT), we reviewed the clinical and molecular data of 216 cases collected in the EWOG-MDS registry, excluding patients

Table 1. Clinical characteristics of patients with JMML and clonal *RAS* mutations surviving long-term without HSCT

Case number	Mutation	Age, y	Sex	Liver, cm	Spleen, cm	WBC, 10^9/L	Mono, 10^9/L	Hb, g/L	Plt, 10^9/L	HbF, %	Outcome (time from diagnosis)
SC047	*NRAS* c.G35T (codon 12 Gly > Val)	0.1	M	0	7	23.7	5.2	102	54	10	alive without HSCT (4.5 y)
I 013	*NRAS* c.G38A (codon 13 Gly > Asp)	1.7	M	2	2	22.2	2.4	103	40	1.3	alive without HSCT (5.5 y)
D 175	*NRAS* c.G35C (codon 12 Gly > Ala)	0.7	F	4	7	18.6	1.3	96	75	3.1	alive without HSCT (8.8 y)
D 028	*NRAS* c.G35A (codon 12 Gly > Asp)	0.4	M	3	5	57.4	8.0	112	192	8.3	alive without HSCT (21.5 y)
CZ011	*NRAS* c.G35A (codon 12 Gly > Asp)	0.5	M	5	2	57.6	11.5	100	162	6.0	dead without HSCT (3.3 y)
D 278	*KRAS* c.C181A (codon 61 Gln > Lys)	0.5	M	2	4	62.5	13.1	87	68	n.d.	alive with HSCT (7.0 y), HSCT given at 3.3 y from diagnosis
Matsuda 9	*NRAS* c.G34A (codon 12 Gly > Ser)	0.8	M	4	5	29.4	4.9	105	113	0.5	alive without HSCT (4.2 y)
Matsuda 10	*NRAS* c.G34A (codon 12 Gly > Ser)	0.8	M	5	10	31.8	6.4	54	100	1.7	alive without HSCT (3.5 y)
Matsuda 11	*KRAS* c.G34A (codon 12 Gly > Ser)	0.3	F	4	1	21.2	1.7	110	52	8.8	alive without HSCT (2.5 y)

Liver and spleen sizes are given in cm below the costal margin. Leukemic clones in all patients had a normal karyotype.
WBC indicates white blood cell count; Mono, monocytes; Plt, platelet count; Hb F, fetal hemoglobin; and nd, not done.