Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY

EAACI

Allergy

ORIGINAL ARTICLE EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Attenuated allergic airway inflammation in *Cd39* null mice

M. Idzko[1,*], C. K. Ayata[1,*], T. Müller[1], T. Dürk[1], M. Grimm[1], K. Baudiß[1], R. P. Vieira[1], S. Cicko[1], C. Boehlke[2], A. Zech[1], S. Sorichter[1], J. Pelletier[3], J. Sévigny[3,4] & S. C. Robson[5]

[1]Department of Pneumology, University Medical Center; [2]Department of Nephrology, University Medical Center, Freiburg, Germany; [3]Centre de recherche en Rhumatologie et Immunologie, Centre Hospitalier Universitaire de Québec; [4]Département de microbiologie-infectiologie et d'immunologie, Faculté de Médecine, Université Laval, Québec, QC, Canada; [5]Department of Medicine, Transplant Institute and Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

To cite this article: Idzko M, Ayata CK, Müller T, Dürk T, Grimm M, Baudiß K, Vieira RP, Cicko S, Boehlke C, Zech A, Sorichter S, Pelletier J, Sévigny J, Robson SC. Attenuated allergic airway inflammation in *Cd39* null mice. *Allergy* 2013; **68**: 472–480.

Keywords
asthma; CD39 ectonucleotidase; chemotaxis; dendritic cells; T-cell priming.

Correspondence
Dr Marco Idzko, Department of Pneumology, University Hospital Freiburg, Killianstrasse 5, 79106, Freiburg, Germany.
Tel.: 00 49 761 270 37310 / 63630 / 63330
Fax: 00 49 761 270 74370
E-mail: marco.idzko@uniklinik-freiburg.de

*These authors contributed equally to the work.

Accepted for publication 21 December 2012

DOI:10.1111/all.12119

Edited by: Hans-Uwe Simon

Abstract

Background: Extracellular Adenosine-5′-Triphosphate (ATP) is known to accumulate in the lung, following allergen challenge, and contributes via activation of purinergic receptors on dendritic cells (DC), to the development of allergic airway inflammation (AAI). Extracellular ATP levels in the airways are normally tightly regulated by CD39. This ectonucleotidase is highly expressed by DC purified from skin (Langerhans cells) and bone marrow, and has been shown to modulate DC adaptive/haptenic immune responses. In this study, we have evaluated the impact of Cd39 deletion and associated perturbation of purinergic signaling in AAI.

Methods: Standard ovalbumin (OVA)–alum and house dust mite (HDM) bone marrow-derived DC (BMDC)-dependent models of AAI were used to study effects of Cd39. Migration assays, time lapse microscopy, and T-cell priming assays were further used to determine functional relevance of Cd39 expression on BMDC in the setting of immune and Th2-mediated responses in these models.

Results: *Cd39*$^{-/-}$ mice exhibited marked increases in BALF ATP levels but paradoxically exhibited limited AAI in both OVA–alum and HDM models. These pathophysiological abnormalities were associated with decreased myeloid DC activation and chemotaxis toward ATP, and were linked to purinergic receptor desensitization responses. Further, *Cd39*$^{-/-}$ DCs exhibited limited capacity to both prime Th2 responses and form stable immune synaptic interactions with OVA-transgenic naïve T cells.

Conclusions: *Cd39*-deficient DCs exhibit limited capacity to induce Th2 immunity in a DC-driven model of AAI *in vivo*. Our data demonstrate a role of CD39 and perturbed purinergic signaling in models of AAI.

Asthma is an important clinical condition, characterized by chronic inflammatory disease of the airways. This disease is associated with variable and recurring symptoms such as reversible airflow obstruction, bronchial hyper-reactivity (BHR) to unspecific stimulus, excessive mucus production, and bronchospasm. Increased Th2 responsiveness and subsequent involvement of mast cells, eosinophils, NK T cells and dendritic cells (DC) contribute to the persistent inflammatory state in the airways, leading to extensive tissue remodeling (1). Compelling findings point to important roles for the myeloid DC (mDC) in the initiation phase and maintenance of allergic airway inflammation (AAI) (2–5).

Recently, extracellular nucleotides (such as Adenosine-5′-Triphosphate (ATP)) – also via activation of the inflammasome pathway (6) – have gained attention as important

Abbreviations
AAI, Allergic airway inflammation; ATP, Adenosine-5′-Triphosphate; BALF, bronchoalveolar lavage fluid; P2R, purinergic receptors; BHR, bronchial hyper-reactivity; BMDC, bone marrow-derived dendritic cell; CD39/ENTPD1, ectonucleoside triphosphate diphosphohydrolase-1; HDM, house dust mite (Dermatophagoides pteronyssinus); MLN, mediastinal lymph node; OVA, ovalbumin.

mediators in AAI. Following allergen challenge, extracellular ATP accumulates in the lungs of asthmatic patients as well as animals in experimental models of AAI. In these latter settings, interference with ATP/type 2 purinergic receptor (P2R) pathways has been shown to attenuate AAI (4, 6, 7).

Extracellular nucleotide levels are tightly regulated by CD39 (ectonucleoside triphosphate diphosphohydrolase-1/ENTPDase1) (8), the dominant vascular, and immune cell ectonucleotidase in the lung and at other sites. CD39 catalyzes the phosphohydrolysis of ATP and ADP to AMP (9), which is then, in turn, metabolized by ecto-5′-nucleotidase to adenosine (10).

Compelling data indicate important roles of CD39 in the regulation of immune function (11–16). Interestingly, *Cd39* deficiency can be linked to both attenuation, for example experimental hepatitis or pancreatitis (15, 16), or worsening of inflammatory diseases *in vivo*, for example transplant rejection (17), experimental diabetes (18), and colitis (19), depending on the innate or adaptive immune response, cellular signaling, and kinetics of responsiveness. Furthermore, inhibition and/or deficiency of *Cd39* results in decreased migration capacity of neutrophils and monocytes/macrophages, perturbation of regulatory T cell, and/or Langerhans cellular function (12, 13, 20, 21).

The importance of CD39 functionality in AAI remains unexplored. Our aim in this study was to elucidate the role of mDC-expressed CD39 in these systems. We demonstrate here for the first time that $Cd39^{-/-}$ mice exhibit attenuated AAI and that this end result is linked to perturbation of ATP-induced migration of mDC *in vivo* with aberrant ovalbumin (OVA)-specific T-cell-mediated priming *in vitro*.

Material and methods

Mice

Female C57BL/6, $Cd39^{-/-}$, and $Cd39^{+/+}$ littermates (17) and OVA-Tcr transgenic OT-II mice, all on a C57BL/6 background, were bred at the University Freiburg. All experiments were performed according to institutional guidelines of the animal ethics committee from the City of Freiburg.

OVA–alum model of allergic airway inflammation

Mice were sensitized to OVA (Worthington Biochemical) via intraperitoneal (i.p.) injection on days 0 and 7 and challenged with OVA aerosols (1% OVA in PBS) on days 19–21, for 30 min (3) (6, 7). On day 22, BALF and mediastinal lymph node (MLN) were collected, followed by lung resection and storage in OCT freezing medium.

Determination of bronchial hyper-responsiveness

Dynamic resistance and compliance to increasing concentrations of aerosolized methacholine (0, 3 mg/ml, 10 mg/ml, 30 mg/ml, and 100 mg/ml) were measured in anesthetized, intubated, and mechanically ventilated (Minivent 485, Hugo-Sachs, March, Germany) mice as previously described (6, 7).

House dust mite induced allergic airway inflammation

Wildtype (WT) and $Cd39^{-/-}$ mice were anesthetized and injected intratracheally (i.t.) with either 100 μg house dust mite (HDM) extract (Greer Laboratories) or vehicle (PBS) as a negative control on day 0 and day 7. On day 14, animals were challenged with an i.t. injection of HDM (100 μg). Animals were assessed for the classical features of AAI such as airway hyper-responsiveness, inflammation, and remodeling and cytokines levels of HDM-restimulated MLN cells on day 17, as previously described (7, 22).

Generation of bone marrow-derived dendritic cells

Dendritic cells were prepared as previously described (7). Briefly, bone marrow cells from WT and $Cd39^{-/-}$ mice were grown in RPMI 1640 with 10% FCS (Biocell Laboratories), 1% gentamicin, 2-mercaptoethanol, and recombinant murine GM-CSF (200 IU/ml). On days 3, 6, and 8, the medium was refreshed. FACS analysis showed that the purity of bone marrow-derived dendritic cell (BMDCs) was greater than 90%.

AAI and adoptive transfer of bone marrow-derived DCs from WT and $Cd39^{-/-}$

WT and $Cd39^{-/-}$ mice were anesthetized and received an i.t. instillation of 1×10^6 vehicle-WT-DCs, OVA-WT-DCs, vehicle-$Cd39^{-/-}$ DCs, or OVA-$Cd39^{-/-}$ DCs, respectively, as described (6) (see Supporting Information). On days 10–12, mice were exposed to OVA aerosols (30 min). Mice were killed 24 h after the last aerosol.

Migration assay *in vivo*

Eighty microlitre of Alexa-488-labeled -OVA (10 mg/ml), with or without ATP (100 μM), was administered i.t. to $Cd39^{-/-}$ and WT mice. Control mice received 80 μl of vehicle. At 24–36 h after injection, migrating DCs were enumerated in the lung and mediastinal LN as $CD11c^+$ $MHCII^+$ cells carrying Alexa-488-labeled material.

Quantitative PCR

Quantitative PCR was performed on LightCyler 480 (Roche) using the fast-blue + UNG kit (Eurogentec). *Cd39* and *ß2-microglobulin* primers/probes were designed using Beacon Designer v7.50 (Premier Biosoft). Percent *reference gene* (*RG*) values for the *gene of interest* (*GOI*) were calculated using the formula: $\%RG = 100 \times 2^{(-\Delta Ct)}$. Cumulative standard deviations were calculated using the formula: $SD = 100 \times 2^{(-\Delta Ct)} \times ((\ln 2 \times SD_{RG})^2 + (\ln 2 \times SD_{GOI})^2)^{1/2}$ (23).

Histology and immunohistochemistry

4 μm thick frozen lung sections were stained with hematoxylin and eosin for histological analysis. Immunohistochemistry for CD39 was carried out with a guinea-pig

anti-mouse antibody as previously described (24, 25) (http://ectonucleotidases-ab.com). For detection, biotinylated anti-guinea-pig secondary antibody was used with Vectastin Elite ABC Kit (Vector Laboratories).

Flow cytometry

Bronchoalveolar lavage cells were stained with I-Ad/I-Ed FITC, CCR3 PE, CD3 and CD19 PE/Cy5, and CD11c APC antibodies. Differential cell counts were analyzed by flow cytometry as previously described (6, 7). For analysis of DC maturation, bone marrow cells were stained with FITC-labeled anti-I-Ad/I-Ed, PE-labeled anti-CD40, anti-CD80, anti-CD83, and anti-CD86, and APC-labeled anti-CD11c antibodies (6, 7). Dead cells were excluded using propidium iodide. Acquisition was performed on a FacsCalibur flow cytometer, and the analysis was made using FlowJo software.

ATP and cytokine measurements in BALF

The BALF ATP level and cytokine concentrations (IFN-γ, IL-4, IL-5, and IL-13) were measured using by ATPLite (Perkin Elmer) or ELISA kits (R&D Systems, Minneapolis, USA), respectively, as previously described (4).

Immune synapse formation and time lapse microscopy

Ovalbumin- or PBS-pulsed WT and $Cd39^{-/-}$ BMDC were stained with FITC-labeled anti-MHCII antibody and plated. Four hours later, purified $CD4^{+}$-OT-II-T cells that were stained with anti-TCR antibody followed by streptavidin-Cy5 were added. The imaging started after 2 h and images have been taken every 5 min. The ZEISS Cell Observer inverted microscope with incubation chamber and Axiovision software (Zeiss) was used for imaging and Imaris software (Bitplane AG, Zurich, Switzerland) for synapse analysis (see Supporting Information).

Results

Lung CD39 expression is down-regulated in AAI

Ovalbumin-alum and HDM-driven AAI was induced in wild-type mice. One day (OVA) or three days (HDM) after the last allergen challenge, mice were euthanized and the expression of CD39 in the lungs was determined by qPCR and IHC. In both models, the induction of AAI was associated with substantial down-regulation of CD39 expression on mRNA (Fig. 1A–B) and protein level (Fig. 1C–D).

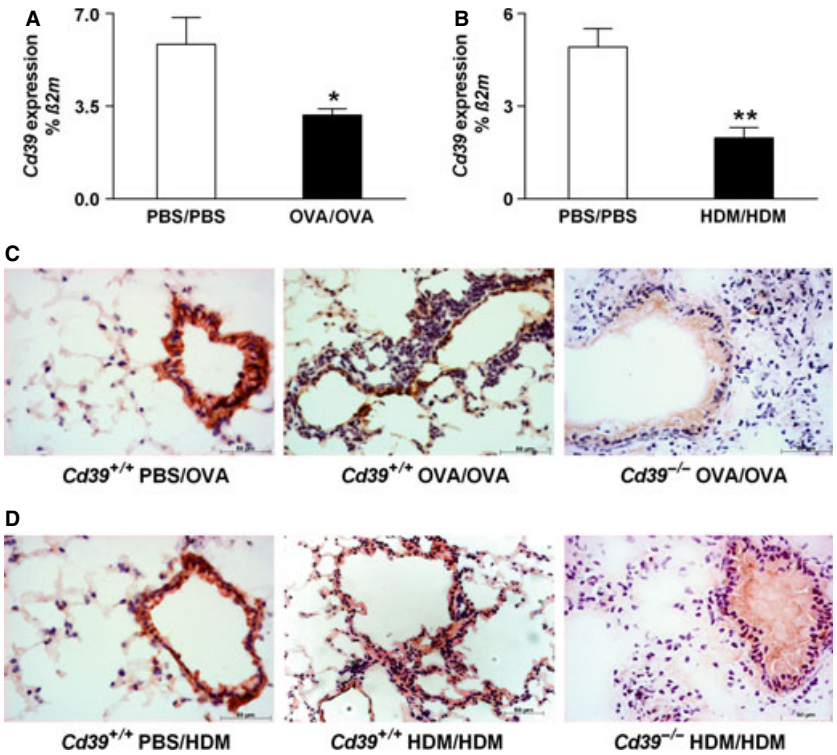

Figure 1 CD39 expression is down-regulated in ovalbumin (OVA)–alum- and house dust mite (HDM)-driven allergic airway inflammation (AAI). In both AAI models, expression of CD39 is decreased at both mRNA (A-B) and protein (C-D) levels, as determined by qPCR and IHC, respectively. Numbers of mice: 6–8 per group $*P < 0.01$, $**$ $P < 0.001$.

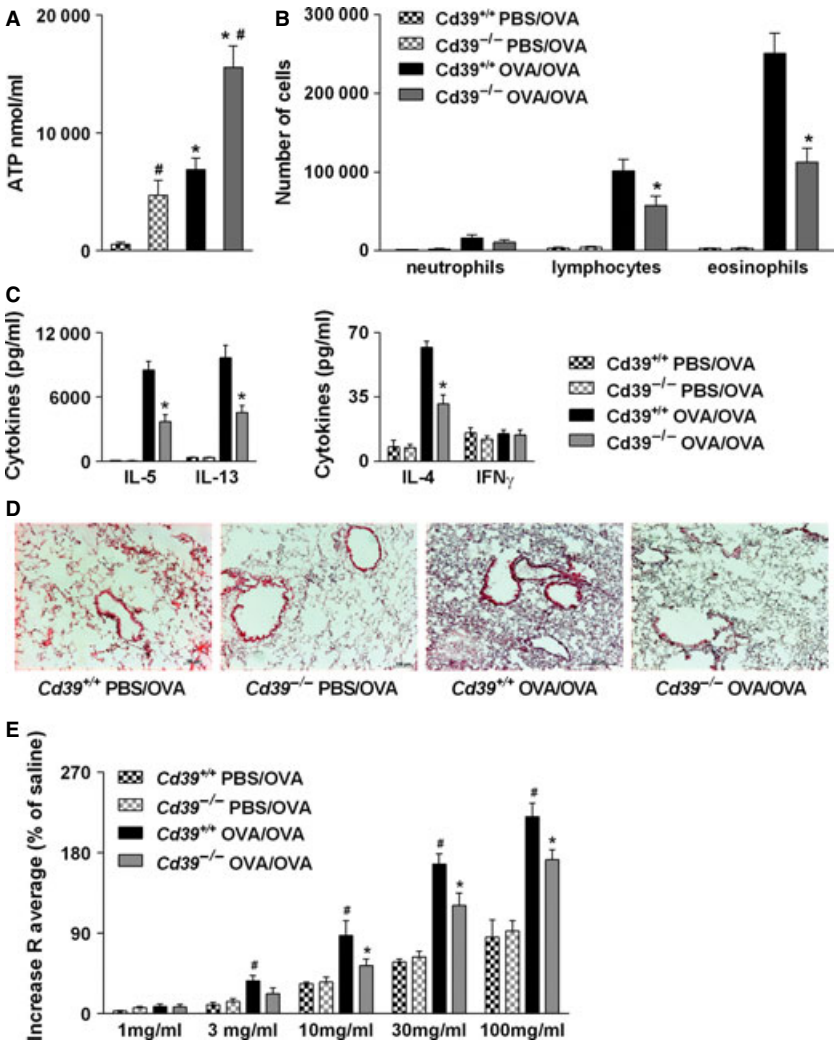

Figure 2 *Cd39*$^{-/-}$ animals are partially protected against OVA–alum-induced AAI. (A) BALF Adenosine-5′-Triphosphate (ATP) level and (B) BALF cell differential counts of WT and *Cd39*$^{-/-}$ mice. (C) Cytokine production by MLN cells restimulated with OVA and quantified by ELISA. Mean of 6–8 mice per group: $^{\#}P < 0.01$ *Cd39*$^{+/+}$ PBS/OVA and *Cd39*$^{+/+}$ OVA/OVA *vs* *Cd39*$^{-/-}$ PBS/OVA and *Cd39*$^{-/-}$ OVA/OVA animals; $^{*}P < 0.01$ *Cd39*$^{+/+}$ OVA/OVA *vs* *Cd39*$^{-/-}$ OVA/OVA. (D) HE staining of lung sections. (E) Bronchial hyper-responsiveness (BHR) to various doses of methacholine measured by changes in resistance [R]. Mean of 6–8 mice per group: $^{\#}P < 0.01$ *Cd39*$^{+/+}$ and *Cd39*$^{-/-}$ PBS/OVA animals *vs* *Cd39*$^{+/+}$ and *Cd39*$^{-/-}$ OVA/OVA animals; $^{*}P < 0.05$ Cd39$^{+/+}$ OVA/OVA *vs* *Cd39*$^{-/-}$ OVA/OVA.

Cd39 deficiency attenuates AAI

Cd39$^{-/-}$ and WT mice were OVA or sham sensitized, followed by OVA challenge, as previously described (7). OVA sensitization, but not sham sensitization, in WT animals resulted in an increase in BALF ATP levels (Fig. 2A), BALF eosinophilia (Fig. 2B), enhanced Th2 cytokine production by MLN cells (Fig. 2C), peribronchial inflammation (Fig. 2D), and BHR to methacholine (Fig. 2E–F).

Somewhat surprisingly, despite increased intrapulmonary ATP levels and the observed down-regulation of CD39 during AAI, *Cd39*$^{-/-}$ mice presented a milder asthma phenotype as seen by significant decrease in all cardinal features of asthma.

Also in the HDM-driven model of AAI, despite the increased pulmonary ATP levels, *Cd39*$^{-/-}$ mice showed a milder phenotype of AAI, as determined by BAL eosinophilia, Th2-cytokine levels lung tissue infiltration, collagen deposition, mucus hyperproduction, and BHR to methacholine (Fig. 3A–E).

Cd39$^{-/-}$ DC and ATP-induced migration *in vitro* and *vivo*

Defective ATP-induced migration in *Cd39*$^{-/-}$ neutrophils and monocytes/macrophages (20) has been observed. The migratory capacity of endogenous WT and *Cd39*$^{-/-}$ mDCs toward ATP (100 μM) *in vivo* was addressed in these studies. ATP-induced recruitment of blood DCs to the lungs was significantly decreased in *Cd39*$^{-/-}$ compared with WT animals (Fig. 4A). In

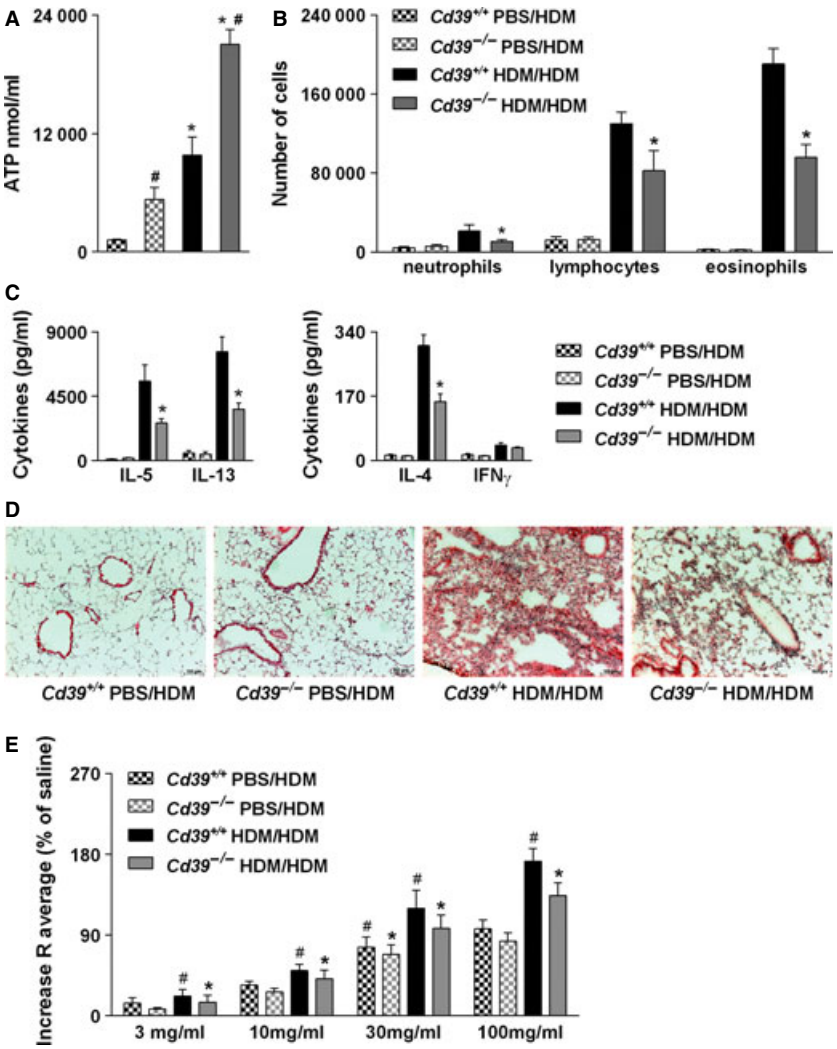

Figure 3 *Cd39* deficiency in HDM-induced model of AAI. (A) BALF ATP level and (B) BALF cell differential counts of WT and $Cd39^{-/-}$ mice. (C) Th2 cytokine production by MLN cells restimulated with HDM and quantified by ELISA. (D) HE staining of lung sections. (E) Bronchial hyper-responsiveness (BHR) to various doses of methacholine, as measured by changes in resistance [R]. Mean of 6–8 mice per group $*P < 0.05$: $^{\#}$ $P < 0.01$ $Cd39^{+/+}$ PBS/HDM and $Cd39^{+/+}$ HDM/HDM *vs* $Cd39^{-/-}$ PBS/HDM and $Cd39^{-/-}$ HDM/HDM animals; $*P < 0.01$ $Cd39^{+/+}$ HDM/HDM *vs* $Cd39^{-/-}$ HDM/HDM.

addition, in *in vitro* experiments, immature BMDCs derived from $Cd39^{-/-}$ show significantly decreased migration capacity, when compared with immature BMDC generated from WT animals (Fig. 4B).

$Cd39^{-/-}$ DC show decreased capacity for priming Th2 immunity *in vivo*

To test the involvement of CD39 in Th2 cell priming capacity of DC, OVA-specific OT-II T cells were injected intravenously into WT mice followed by an i.t. administration of OVA- or PBS-pulsed WT or $Cd39^{-/-}$ BMDCs. Mice receiving OVA-pulsed $Cd39^{-/-}$ DCs showed significant lower levels of the Th2 cytokines (IL-4, IL-5, IL-13) in the MLN, as compared to mice receiving OVA-pulsed WT DCs (Fig. 5A).

To elucidate the relevance in context of AAI, we next used a DC-driven model of AAI. I.t. application of OVA-pulsed WT DCs, but not PBS-pulsed WT DCs, into WT animals leads to BAL eosinophilia, peribronchial and perivascular inflammation, and Th2 cytokines production by restimulated MLN cells (Fig. 5B–D). Of note, in animals receiving OVA-pulsed $Cd39^{-/-}$ DCs, all classical features of AAI were significantly decreased.

In contrast, OVA-pulsed WT DCs were able to readily sensitize $Cd39^{-/-}$ animals (Fig. 5E–F). These suggest that specific expression of CD39 on DC alone contributes in the sensitization phase but not during the effector phase of AAI.

The observed limitation in Th2 priming appeared to be not due to a defect in OVA-induced maturation of $Cd39^{-/-}$ DC, as assessed by the surface expression of CD40, CD83, CD80, and CD86 (data not shown).

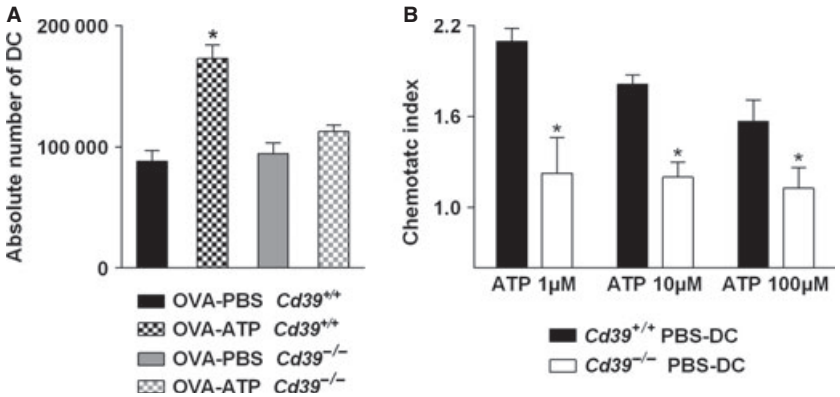

Figure 4 Unresponsiveness of blood *Cd39*$^{-/-}$ dendritic cells (DC) in migrating toward ATP *in vivo*. (A) Effect of ATP on the absolute number of DCs in the lungs of WT and *Cd39*$^{-/-}$ animals. (B) Immature bone marrow-derived dendritic cell (BMDC) from *Cd39*$^{-/-}$ show significantly decreased capacity to migrate toward ATP *in vitro*. Mean of six mice/group $*P < 0.05$.

CD39 expression on DCs is involved in regulating synapse formation with T cells

Finally, the involvement of CD39 expression on DC in the formation of dendrites and in establishing stable interactions with T cells was studied. The time of interaction between purified CD4 + OT-II cultured with OVA-pulsed *Cd39*$^{-/-}$ DC was significantly shorter, as compared to OVA-pulsed WT-DC and OT-II (Fig. 5G).

Discussion

Compelling studies point to an important role of extracellular nucleotides and associated ectonucleotidases, such as CD39, in regulating immune responses (13). Deletion of *Cd39*, resulting in an imbalance between extracellular nucleotides and nucleosides, has been shown to adversely impact the outcomes of several acute and chronic inflammatory disorders, for example DSS-induced colitis (19), renal ischemia-reperfusion injury (26), and diabetic nephropathy (27), among others. These and other studies suggest that augmenting CD39-associated NTPDase activity might be a useful therapeutic strategy in such inflammatory states.

Recently, we reported that allergen challenge of asthmatics and sensitized mice results in accumulation of airway ATP levels, which then acts via activation of P2 receptors and contributes to cardinal features of AAI (4, 7). CD39 hydrolyzes the proinflammatory molecule ATP to initiate an enzymatic cascade that ultimately produces the anti-inflammatory molecule adenosine via CD73 (11), and a down-regulation of CD39-expression/activity has been observed in inflamed tissues (13) including AAI (Fig. 1). Therefore, one might speculate that *Cd39* deficiency would in fact aggravate AAI.

Here, we report that despite increased BALF ATP levels, *Cd39* deficiency is associated with amelioration of all cardinal features of AAI in mice, including eosinophilic airway inflammation, goblet cell hyperplasia, Th2 cytokine production, and BHR to inhaled methacholine.

This unexpected result could be partially explained by aberrant functions and impaired migration of blood DC toward ATP. Recently, we reported that ATP contributes to the recruitment of DCs to the lung during AAI via P2Y2R signaling. The migration of immature blood DC to lung and then subsequently to the MLN is an integral part of both primary and secondary immune response to inhaled allergens (5). Consequently, in a manner similar to previously reported compounds that interfere with the migration capacity of DC (3), AAI is substantively decreased in P2Y2R$^{-/-}$ animals (4, 7). While the migration of skin DC to draining lymph nodes in the model of contact dermatitis is not impacted in *Cd39*$^{-/-}$ mice (21), recent studies suggest a more general disordered ATP-induced migration of monocytes and macrophages in these animal. This phenotype has been linked to desensitization of P2YR subtypes (20, 21). Therefore, our observation that *Cd39*$^{-/-}$ DCs were unable to migrate in response to ATP *in vivo* and *in vitro* also suggests desensitization of select P2YR in *Cd39*$^{-/-}$ DCs that impact AAI.

During the sensitization of AAI phase, mDCs play a crucial role is the priming of naïve T cells into allergen-specific Th2 effector cells. Interestingly, Th2 cell differentiation in the MLN following intratracheal injection of OVA-pulsed *Cd39*$^{-/-}$ DCs is impaired, suggesting the involvement of CD39 in priming of naïve OT-II T cells. These observations are in line with a previous report by us, demonstrating an impaired ability of *Cd39*$^{-/-}$ DCs to stimulate hapten-reactive T cells (21). This defect is associated with resistance of *Cd39*$^{-/-}$ mice to the development of delayed contact hypersensitivity.

To further elucidate a potential involvement of CD39 on DCs in T-cell priming during AAI, OVA-pulsed WT and *Cd39*$^{-/-}$ DCs were adoptively transferred into the lungs of naïve animals, followed by OVA challenge. In parallel with this hypothesis, WT animals receiving OVA-pulsed *Cd39*$^{-/-}$ DCs showed a significant reduction in cardinal features of AAI as compared to animals receiving OVA-pulsed WT DCs.

The induction and maintenance of Th2 immunity in the lungs depend upon adequate migration and antigen-processing function in association with maturation of lung DCs (2). However, in accordance with previous observations, the

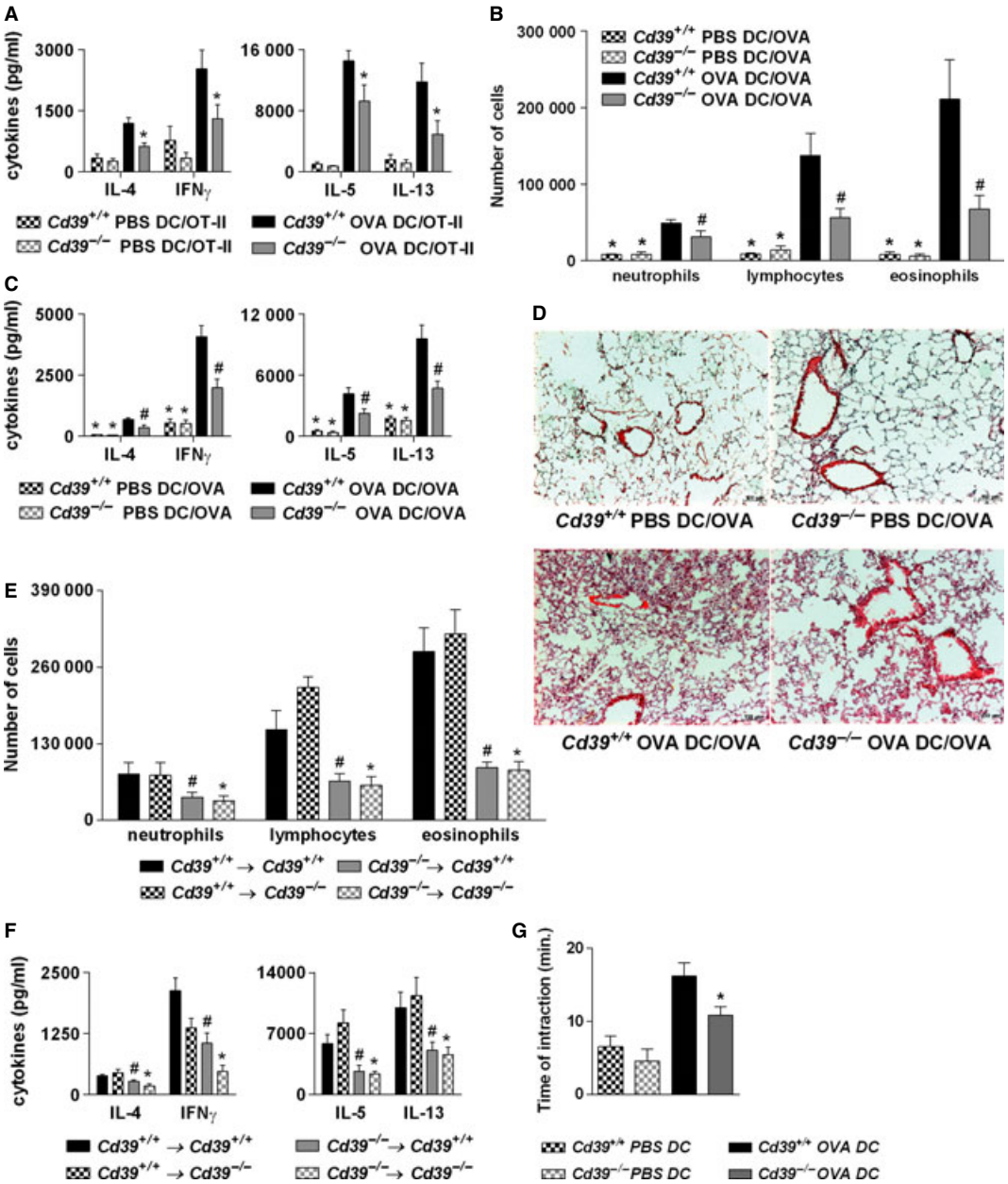

Figure 5 Effect of CD39 on T-cell priming capacity and synapse formation of DCs *in vivo* and *in vitro*. (A) Mice were instilled i.t. with PBS- or OVA-pulsed WT or *Cd39*$^{-/-}$ BMDC; on day two, OVA-specific naïve CD4^{+} T cells were injected i.v. On day 6, MLN cells were isolated and cultured for 4 days. Levels of IL-4, IL-5, and IL-13 in the supernatants were determined by ELISA. Data are mean ± SEM, $n = 6$ animals per group $^{*}P < 0.05$. (B) BALF cell differential counts of WT and CD39$^{-/-}$ mice received OVA-pulsed WT or *Cd39*$^{-/-}$ DCs i.t. (C) Production of Th2 cytokines (IL-4, IL-5, IL-13) in the MLN. (D) HE staining of lung sections. (E, F) Sensitization of *Cd39*$^{-/-}$ animals by OVA-pulsed WT DCs. Mean of 6–8 mice/group $^{*}P < 0.05$ PBS-DCs *vs* OVA-DCs; $\#P < 0.05$ WT-OVA-DCs *vs* *Cd39*$^{-/-}$ OVA-DCs. (G) Immune synapse formation between WT/*Cd39*$^{-/-}$ BMDCs and CD4^{+} T cells from OT-II mice; $^{*}P < 0.05$.

OVA-induced expression of CD40, CD80, CD83, CD86, and MHCII did not differ between immature or mature BMDCs from WT and *Cd39*$^{-/-}$ animals.

The immune synapse is considered as a distinct extracellular space shared by a lymphocyte and DC, which is closely linked to antigen presentation. This special junctional structure serves as a microenvironment where various molecule clusters interact with each other leading to the activation of the T cell, based on the pairing of TCR and presented antigen. Using time lapse imaging, we observe a significant shorter time of interaction between OTII T cells and OVA-*Cd39*$^{-/-}$ DCs compared with WT-DCs, while the total

number of synapse formation did not differ. The formation of the immune synapse is equally important as the proper duration of the interaction, allowing full-scale activation of the T cell upon antigen recognition. In fact, both parameters are strictly dependent on capabilities of the DCs (28). There is increasing evidence concerning autocrine and paracrine involvement of ATP in modulating cellular functions. ATP release, leading to the activation of P2 receptors, supports the establishment of a basal stimulation status that facilitates subsequent activation and regulation of many signal transduction pathways (29).

Recent studies suggest that ATP-P2R signaling within the immune synapse plays a role in T-cell activation upon antigen representation (30, 31). In this context, involvement of ATP binding to P2X1R and P2X4R subtypes has been shown as a part of T-cell activation process (32).

Previous studies have reported that P2R subtypes are prone to ATP-mediated desensitization (33). Further, CD39 is palmitoylated and subsequently localized to lipid rafts that comprise and regulate immune synapse (34, 35). These data suggest that antigen presentation occurs under specific ATP concentrations regulated by CD39, P2 receptors, and Pannexin-1 within or adjacent to the immune synapse. Thus, our data might indicate that contributions of DC-expressed CD39 in the regulation of the ATP/ADP levels within the immune synapse may be crucial in the determination of the activation status of the T cells.

In summary, we demonstrate that genetic deletion of *Cd39* is associated with decreased AAI in mice. Absence of CD39 is associated with aberrant P2 signaling, which modulates DC migration and limits the capacity of these cells to prime Th2 responses *in vivo*.

Financial support

This study was supported by a grant to M.I. from the German Research Foundation (DFG, ID 7/4-3). JS acknowledges support from the Canadian Institutes of Health Research, and he is also a recipient of a Senior Scholarship from the *Fonds de recherche Santé Québec*. SCR acknowledges support from NHLBI, NIH R01 HL094400, and P01 HL087203.

Author contributions

Each named author contributed to the design of the study, its implementation, and/or the writing of the manuscript.

Conflict of interest

None of the authors has a conflict of interest with respect to the study.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary methods.

References

1. Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;**17**:255–281.
2. Idzko M et al. Local application of FTY720 to the lung abrogates experimental asthma by altering dendritic cell function. *J Clin Invest* 2006;**116**:2935–2944.
3. Idzko M et al. Inhaled iloprost suppresses the cardinal features of asthma via inhibition of airway dendritic cell function. *J Clin Invest* 2007;**117**:464–472.
4. Idzko M et al. Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 2007;**13**:913–919.
5. Lambrecht BN, Hammad H. Lung dendritic cells in respiratory viral infection and asthma: from protection to immunopathology. *Annu Rev Immunol* 2012;**30**:243–270.
6. Muller T et al. A potential role for P2X7R in allergic airway inflammation in mice and humans. *Am J Respir Cell Mol Biol* 2011;**44**:456–464.
7. Muller T et al. The purinergic receptor P2Y2 receptor mediates chemotaxis of dendritic cells and eosinophils in allergic lung inflammation. *Allergy* 2010;**65**: 1545–1553.
8. Burch LH, Picher M. E-NTPDases in human airways: regulation and relevance for chronic lung diseases. *Purinergic Signal* 2006;**2**:399–408.
9. Kukulski F et al. Comparative hydrolysis of P2 receptor agonists by NTPDases 1, 2, 3 and 8. *Purinergic Signal* 2005;**1**:193 –204.
10. Fausther M et al. Coexpression of ecto-5′-nucleotidase/CD73 with specific NTPDases differentially regulates adenosine formation in the rat liver. *Am J Physiol Gastrointest Liver Physiol* 2012;**302**: G447–G459.
11. Robson SC, Sevigny J, Zimmermann H. The E-NTPDase family of ectonucleotidases: structure function relationships and pathophysiological significance. *Purinergic Signal* 2006;**2**:409–430.
12. Corriden R et al. Ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1/CD39) regulates neutrophil chemotaxis by hydrolyzing released ATP to adenosine. *J Biol Chem* 2008;**283**:28480–28486.
13. Deaglio S, Robson SC. Ectonucleotidases as regulators of purinergic signaling in thrombosis, inflammation, and immunity. *Adv Pharmacol* 2011;**61**:301–332.
14. Dwyer KM et al. CD39 and control of cellular immune responses. *Purinergic Signal* 2007;**3**:171–180.
15. Kunzli BM et al. Disordered pancreatic inflammatory responses and inhibition of fibrosis in CD39-null mice. *Gastroenterology* 2008;**134**:292–305.
16. Beldi G et al. Natural killer T cell dysfunction in CD39-null mice protects against concanavalin A-induced hepatitis. *Hepatology* 2008;**48**:841–852.
17. Robson SC et al. Ectonucleotidases of CD39 family modulate vascular inflammation and thrombosis in transplantation. *Semin Thromb Hemost* 2005;**31**: 217–233.
18. Enjyoji K et al. Deletion of cd39/entpd1 results in hepatic insulin resistance. *Diabetes* 2008;**57**:2311–2320.
19. Friedman DJ et al. From the Cover: CD39 deletion exacerbates experimental murine colitis and human polymorphisms increase susceptibility to inflammatory bowel disease. *Proc Natl Acad Sci USA* 2009;**106**: 16788–16793.
20. Goepfert C et al. Disordered cellular migration and angiogenesis in cd39-null mice. *Circulation* 2001;**104**:3109–3115.

21. Mizumoto N et al. CD39 is the dominant Langerhans cell-associated ecto-NTPDase: modulatory roles in inflammation and immune responsiveness. *Nat Med* 2002;**8**:358–365.
22. Hammad H et al. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009;**15**:410–416.
23. Muller PY et al. Processing of gene expression data generated by quantitative real-time RT-PCR. *Biotechniques* 2002;**32**: p. 1372–1374, 1376, 1378-9.
24. Vieira RP et al. Purinergic receptor type 6 contributes to airway inflammation and remodeling in experimental allergic airway inflammation. *Am J Respir Crit Care Med* 2011;**184**:215–223.
25. Martin-Satue M et al. Localization of plasma membrane bound NTPDases in the murine reproductive tract. *Histochem Cell Biol* 2009;**131**:615–628.
26. Lu B et al. The impact of purinergic signaling on renal ischemia-reperfusion injury. *Transplantation* 2008;**86**:1707–1712.
27. Friedman DJ et al. The vascular ectonucleotidase ENTPD1 is a novel renoprotective factor in diabetic nephropathy. *Diabetes* 2007;**56**:2371–2379.
28. Benvenuti F et al. Dendritic cell maturation controls adhesion, synapse formation, and the duration of the interactions with naive T lymphocytes. *J Immunol* 2004;**172**:292–301.
29. Corriden R, Insel PA. Basal release of ATP: an autocrine-paracrine mechanism for cell regulation. *Sci Signal*. **3**: re1.
30. Filippini A, Taffs RE, Sitkovsky MV. Extracellular ATP in T-lymphocyte activation: possible role in effector functions. *Proc Natl Acad Sci USA* 1990;**87**:8267–8271.
31. Schenk U et al. Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels. *Sci Signal*, 2008;1:ra6.
32. Woehrle T et al. Pannexin-1 hemichannel-mediated ATP release together with P2X1 and P2X4 receptors regulate T-cell activation at the immune synapse. *Blood* 2010;**116**: 3475–3484.
33. Enjyoji K et al. Targeted disruption of cd39/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. *Nat Med* 1999;**5**: 1010–1017.
34. Koziak K et al. Palmitoylation targets CD39/endothelial ATP diphosphohydrolase to caveolae. *J Biol Chem* 2000;**275**: 2057–2062.
35. Kittel A et al. CD39 as a caveolar-associated ectonucleotidase. *Biochem Biophys Res Commun* 1999;**262**:596–599.