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The purinergic receptor $P2Y_2$ receptor mediates chemotaxis of dendritic cells and eosinophils in allergic lung inflammation

T. Müller[1]*, B. Robaye[2]*, R. P. Vieira[1], D. Ferrari[3], M. Grimm[1], T. Jakob[4], S. F. Martin[4], F. Di Virgilio[3], J.-M. Boeynaems[2], J. C. Virchow[5] & M. Idzko[1]

[1]Department of Pneumology, University Medical Centre Freiburg, Freiburg, Germany; [2]IRIBHM and Erasme Hospital, Université Libre de Bruxelles, Bruxelles, Belgium; [3]Department of General Pathology, University of Ferrara, Ferrara, Italy; [4]Allergy Research Group, University Medical Centre Freiburg, Freiburg, Germany; [5]Department of Pneumology, University Medical Centre Rostock, Rostock, Germany

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Correspondence
Dr. Marco Idzko, University Medical Centre Freiburg, Department of Pneumology, Killianstrasse 5, 79106 Freiburg, Germany.
Tel.: +49761/270-6363
Fax: +49761/270-6362
E-mail: marco.idzko@uniklinink-freiburg.de

*T. M. and B. R. contributed equally to the work.

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Abstract

Background: Extracellular ATP contributes to the pathogenesis of asthma via signalling at purinergic receptors. However, the precise purinergic receptors subtypes mediating the pro-asthmatic effects of ATP have not been identified, yet.
Methods: *In vivo* studies were performed using the OVA-alum model. Functional expression of the $P2Y_2$ purinergic receptor subtype on human monocyte-derived dendritic cells and eosinophils was investigated using real-time PCR, migration assays, and production of reactive oxygen species.
Results: Compared to wild-type animals $P2Y_2-/-$ mice showed reduced allergic airway inflammation which can be explained by defective migration of blood myeloid DCs towards ATP *in vitro* and *in vivo*, whereas the influence of ATP on maturation and cytokine production was not changed. Additionally, ATP failed to induce migration of bone marrow–derived eosinophils from $P2Y_2$R-deficient animals. The relevance of our findings for humans was confirmed in functional studies with human monocyte-derived DCs and eosinophils. Interestingly, stimulation of human DCs derived from allergic individuals with house dust mite allergen induced functional up-regulation of the $P2Y_2$R subtype. Furthermore, eosinophils isolated from asthmatic individuals expressed higher levels of $P2Y_2$R compared to healthy controls. This was of functional relevance as these eosinophils were more sensitive to ATP-induced migration and production of reactive oxygen metabolites.
Conclusions: In summary, $P2Y_2$R appears to be involved in asthmatic airway inflammation by mediating ATP-triggered migration of mDCs and eosinophils, as well as reactive oxygen species production. Together our data suggest that targeting $P2Y_2$R might be a therapeutic option for the treatment of asthma.

Allergic asthma is one of the most common chronic diseases in Western societies. Its pathogenesis includes mucus hypersecretion, structural remodelling of the airway walls, variable airway obstruction, bronchial hyper-responsiveness (BHR) to nonspecific stimuli, and infiltration of the airway wall with T-helper type 2 (Th2) cells, eosinophils, and mast cells which have all been shown to contribute to asthmatic inflammation. In addition to the above-mentioned cells, myeloid dendritic cells (mDCs) which are able to take up antigens and initiate Th2-dominated immune responses are also essential in asthma pathogenesis (1, 2).

Over the last years, extracellular nucleotides such as adenosine-5′-triphosphate (ATP) have gained attention as mediators of inflammation via the activation of purinergic

Abbreviations
ATP, adenosine-5′-Triphosphate; BHR, bronchial hyper-reactivity; BMDCs, bone marrow–derived dendritic cells; Der p1, house dust mite major allergen 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; HDM, house dust mite; IL, interleukin; mDCs, myeloid dendritic cells; MLN, mediastinal lymph node; OVA, ovalbumin; P2Y2R, purinergic receptor P2Y, subtype 2.

receptors which can be subdivided into metabotropic P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$) and P2X receptors ($P2X_1$-$P2X_7$) which are ligand gated ion channels (3–5). $P2Y_2R$ and $P2Y_{11}R$ are activated by ATP, $P2Y_1R$, $P2Y_{12}R$, and $P2Y_{13}R$ by ADP (6–9). UTP (which is equipotent with ATP at the $P2Y_2R$) is the most potent agonist for the $P2Y_4R$, whereas UDP preferentially binds to $P2Y_6R$ subtypes (10–12). In contrast to P2Y receptors, the only naturally occurring ligand for P2X receptors is ATP (5).

It has recently been shown that ATP, released into the airways during asthmatic airway inflammation, can modulate the function of myeloid dendritic cells (mDCs) thereby triggering and maintaining asthmatic airway inflammation (13). However, the precise P2R subtypes involved have not yet been identified. The aim of this study was to investigate the involvement of the $P2Y_2R$ subtype in allergic airway inflammation. Thereby, we were able to demonstrate that $P2Y_2$ receptors contribute the classical features of asthma via the recruitment of mDCs and eosinophils into the lungs.

Material and methods

Mice

C57/Bl6 WT animals and P2Y2 receptor–deficient mice ($P2Y_2R-/-$) on C57/Bl6 background which were generated as previously described (14) were bred at the University of Freiburg. OVA-TCR transgenic mice (OTII) were purchased from Charles River. For all experiments permission was obtained from the animal ethics committee from the City of Freiburg.

OVA-alum model of allergic airway inflammation

Mice were sensitized to OVA (Worthington Biochemical, Lakewood, NJ, USA) via intraperitoneal injection of OVA/alum (10 μg OVA grade V; Worthington Biochemical Corp.; adsorbed to 1 mg aluminium hydroxide; Thermo Scientific Pierce Imject Alum, Rockford, IL, USA) on days 0 and 7 and were challenged with OVA aerosols on days 19–21, delivered from a jet nebulizer delivering 1%OVA in PBS for 30 min. Twenty-four h after the last OVA exposure, BAL was performed with 3×1 ml of Ca^{2+} and Mg^{2+} free PBS (Invitrogen, Darmstadt, Germany) supplemented with 0.1 mM sodium EDTA, followed by lung resection and storage in OCT freezing medium. To measure cytokine levels, mediastinal LN cells were plated in round bottom 96-well plates (1×10^6 cells/ml) and restimulated with OVA (10 μg/ml) for 4 days. Then, supernatants were collected and the presence of IL-4, IL-5, IL-13, and IFN-γ was assayed by ELISA (R&D systems, Minneapolis, MN, USA).

Migration assay *in vivo*

To address migration of lung DCs, 80 μl of FITC-OVA (10 mg/ml), with or without ATPγS – an ATP analogue which cannot be metabolized to adenosine by endogenous membrane-bound ectonucleotidases – was administered intratracheally to $P2YR_2-/-$ and $P2YR_2+/+$ mice. Control mice received 80 μl of vehicle. At 24–36 h after injection, migrating DCs were enumerated in the mediastinal lymph nodes as CD11c + MHCII + cells carrying FITC + material (13).

Generation of bone marrow–derived dendritic cells (BMDCs)

DCs were prepared as previously described (15). Briefly, bone marrow cells from WT and $P2Y_2R-/-$ C57/Bl6 mice were grown in RPMI 1640 supplemented with gentamycin, 2-mercaptoethanol, 10% FCS (Biocell Laboratories), and recombinant murine GM-CSF (200 IU/ml). On days 3, 6, and 8, the medium was refreshed and GM-CSF was added. The purity of bone marrow–derived DCs was greater than 90%.

On day 9 of culture, cells were pulsed overnight with 100 μg/ml LPS-low OVA (Worthington Biochemicals) in the presence of 100 μM ATP, or 100 μM of the nonhydrolysable ATP analogue ATPγS or PBS. After antigen pulsing, nonadherent DCs were collected, washed to remove free OVA or ATP and resuspended in medium.

Generation of bone marrow–derived eosinophils

Eosinophils were prepared as previously described (16). Briefly, bone marrow cells from WT and $P2Y_2R-/-$ C57/Bl6 mice were grown in RPMI 1640 supplemented with gentamycin, 2-mercaptoethanol, and 20% FCS, in the presence of 100 ng/ml stem cell factor and 100 ng/ml FLT3 ligand (both from Immunotools, Frisoythe, Germany) for 4 days. Then, the medium was replaced with RPMI containing 10 ng/ml rmIL-5 (Peprotech). Cells were cultured for another 4 days, from this point forward medium was replaced every other day. Cells were used for experiments on day 12. The purity of bone marrow–derived eosinophils was greater than 90%, as determined by cytospin and FACS (CCR3 expression).

Human individuals

Blood donors for the generation of human monocyte-derived DCs

Atopic asthmatics allergic to house dust mite were recruited on the basis of airway hyper-responsiveness, positive allergen skin prick tests, elevated total and specific IgE concentrations. Participants gave their written informed consent (for patients characteristic see the table in Data S1). The study was approved by the local ethics committee. Inhaled corticosteroids were withdrawn at least 14 days before blood was taken. None of the patients took systemic corticosteroids.

Blood donors for eosinophils

Eight patients with a diagnosis of allergic asthma were recruited on the basis of airway hyper-responsiveness, positive allergen skin prick tests, elevated total or specific IgE concentrations. Eight subjects without any history of allergic signs or symptoms, a negative skin prick test to a large panel

of allergens and normal IgE levels served as controls (for patients characteristic see the table in Data S1). Participants gave their written informed consent. The study was approved by the local ethics committee. Inhaled corticosteroids were withdrawn at least 14 days before blood was taken. None of the patients took systemic corticosteroids.

Preparation of monocyte-derived DCs

$CD14^+$ monocytes were isolated from 200 ml of peripheral blood, obtained from HDM-asthmatic human volunteers, using Ficoll-Paque gradients and anti-CD14 mAb-coated MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) (17). Isolated cells were resuspended in RPMI 1640 supplemented with gentamycin, 2-mercaptoethanol, and 10% FCS, 1000 U/ml IL-4, 200 ng/ml GM-CSF (Immunotools, Friesoythe, Germany). After 6 days of culture at 37°C in a humidified CO_2 (5%) atmosphere, monocyte-derived immature DCs (iDCs) were >95% $CD1a^+$, $CD80^{low}$, $CD86^{low}$, $CD83^{low}$, and $CD115^{high}$. Further differentiation into mature DCs (mDCs) was induced by treatment with 3 μg/ml lipopolysaccharide (LPS) (*Escherichia coli*, serotype 0111:B4, Sigma, Deisenhofen, Germany) for 48 h. Mature DCs were >95% $CD80^{high}$, $CD86^{high}$, $CD83^{high}$, and $CD115^{low}$. Analysis of cell surface molecules was performed by flow cytometry using FACScan (Becton Dickinson, Heidelberg, Germany).

Isolation of eosinophils

Human eosinophils were isolated from 200 ml of peripheral blood, obtained from asthmatic or healthy human volunteers, using Ficoll-Paque gradients and negative selection with a mixture of anti-CD16 mAb-coated MicroBeads to remove neutrophils (Miltenyi Biotec, Bergisch Gladbach, Germany). Resulting eosinophils were resuspended in PBS, and the purity of isolated cells judged by Pappenheim staining was > 98%.

Migration assay *in vitro*

Experiments were performed in triplicate using 24-well Transwell chambers. Buffer or different concentrations of ATP were added into the lower compartment wells. Human or murine cells (10^5 cells/well) were added to the upper compartment and incubated at 37°C for 90 min in a humidified atmosphere. After removing the cells from the upper compartment, migrated DCs on the lower chamber were fixed in methanol and stained with haematoxylin. Results are shown as chemotactic index, calculated as the number of cells in the lower chamber containing the different stimuli divided by the number of cells in the chamber containing medium alone.

Lucigenin-dependent chemiluminescence

Eosinophils were resuspended to a density of 5×10^4 cells/ml containing 200 μM lucigenin. Measurements were performed in triplicate at 37°C. Reactions over a 60-min time period after cell stimulation were followed and expressed as intensity integral counts (18).

Statistical analysis

If not stated otherwise, groups were compared using ANOVA, followed by Bonferroni comparison test. Probability values of $P < 0.05$ were regarded as significant.

Results

P2Y$_2$R deficiency attenuates allergic airway inflammation

To investigate whether P2Y$_2$ receptor deficiency protects against the development of experimental asthma, we sensitized WT and P2Y$_2$R−/− mice via i.p. injections of OVA in the Th2 adjuvant alum. Control mice were sham sensitized using PBS/alum followed by challenge with OVA aerosols. As expected, OVA-sensitized and challenged WT animals developed broncho-alveolar lavage fluid (BALF) eosinophilia and lymphocytosis (Fig. 1A) accompanied by enhanced Th2 cytokine production by mediastinal lymph node (MLN) cells (Fig. 1B), as well as peribronchial inflammation (Fig. 1C) which was not seen in sham-sensitized mice. P2Y$_2$R deficiency in OVA-sensitized and challenged animals was associated with a mild but significant reduction in lymphocyte and eosinophil numbers in BALF, as well as reduced peribronchial and perivascular tissue infiltration on lung sections. Additionally, production of the Th2-cytokines IL-4, IL-5, and IL-13 by restimulated MLN cells was also decreased in P2Y$_2$R-deficient animals (Fig. 1A–C).

Bronchial hyper-reactivity (BHR) to nonspecific stimuli like methacholine is a characteristic feature of bronchial asthma. BHR was measured 24 h after the last OVA challenge by invasive measurement of dynamic resistance and compliance in ventilated mice (see Data S1). As shown in Fig. 1D, allergen challenge induced a significant change in responsiveness to aerosolized methacholine in OVA-sensitized mice compared with sham-sensitized mice. OVA-sensitized and challenged P2Y$_2$R-deficient mice had a significantly lower dynamic resistance/drop in compliance compared to WT animals.

P2Y$_2$R is required for ATP-induced migration of mDCs *in vitro* and *in vivo*

It has recently been shown that ATP is a chemoattractant for immature bone marrow–derived dendritic cells (BMDCs) *in vitro* and is involved in the recruitment of myeloid DCs to the lungs and MLNs *in vivo* (13). We therefore questioned whether the observed attenuation of allergic airway inflammation in P2Y$_2$R−/− animals might be attributed to the decreased migratory capacity of DCs towards ATP. For this purpose, WT and P2Y$_2$R−/− animals received an i.t. application of OVA-FITC-vehicle, OVA-FITC-ATPγS or vehicle alone. As shown in Fig. 2A, in WT mice concomitant treatment with ATPγS led to an enhanced absolute number of DCs in the lungs; however, this ATP-dependent recruitment of blood DCs to the lungs was not observed in P2Y$_2$R−/− animals. There was no evidence of involvement of the ATP-induced up-regulation of CCL20 in the lung in this response,

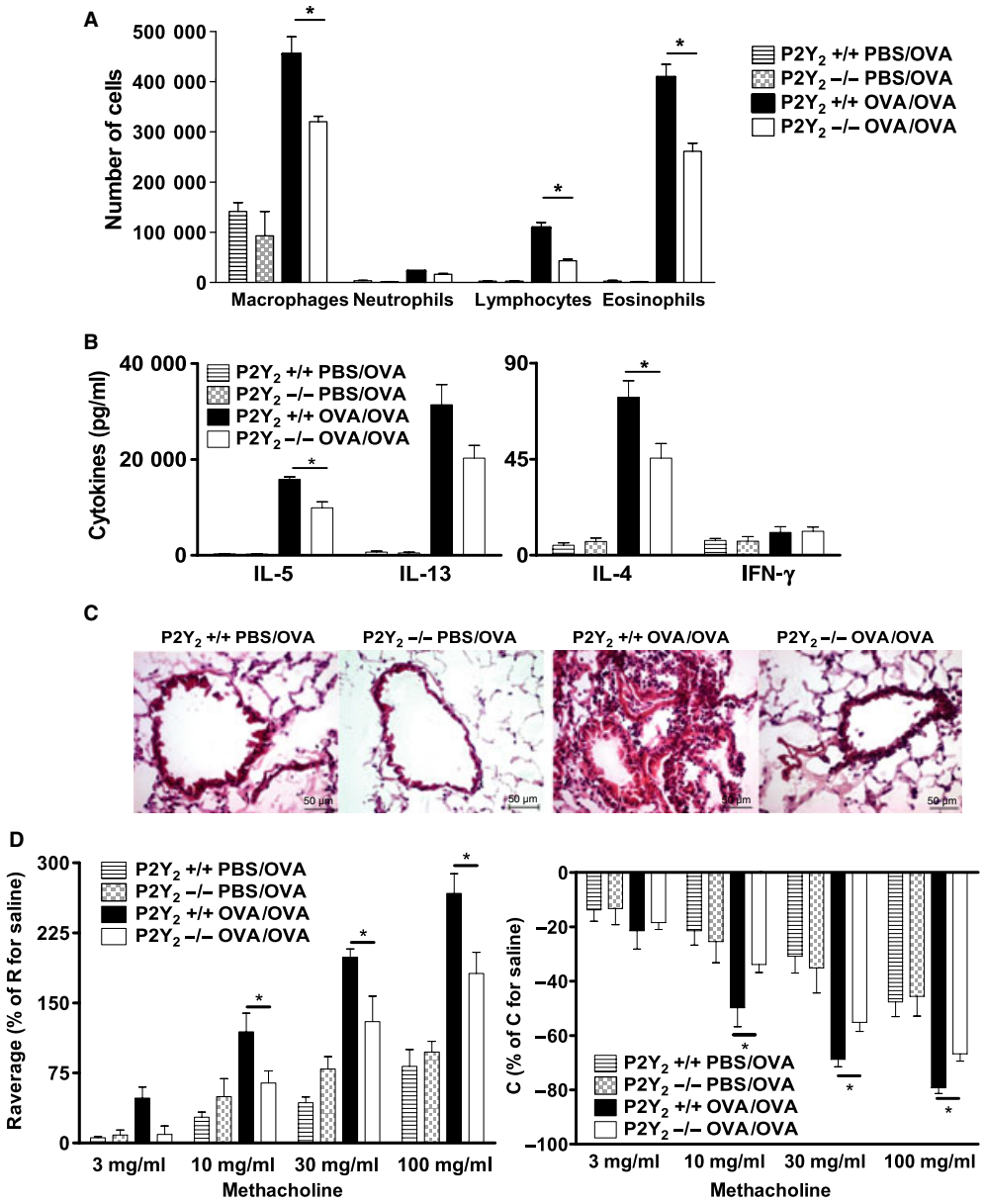

Figure 1 P2Y$_2$R-deficient animals show reduced allergic airway inflammation. (A) BALF cell differential counts of WT and P2Y$_2$R−/− mice. (B) Cytokine production by MLN cells restimulated with OVA measured by ELISA. (C) HE staining of lung sections (D) Bronchial hyper-responsiveness (BHR) to various doses of methacholine measured by changes in resistance (R) and lung compliance (C). Mean of 6–8 mice per group $*P < 0.05$.

as this was similar between WT and P2Y$_2$R−/− animals (Fig. 2B). However, *in vitro* experiments revealed that in contrast to BMDCs derived from WT mice immature BMDCs generated from P2Y$_2$R−/− animals do not migrate in response to ATP (Fig. 2C).

In addition, the number of MHCII + CD11c + FITC + DCs reaching the MLNs in P2Y$_2$−/− animals was also reduced compared to WT animals (Fig. 2D). To exclude that this was because of the altered CCR7 expression in P2Y$_2$R−/− animals, BMDCs were stimulated *in vitro* with ATP ± OVA and CCR7 expression was analysed by real-time PCR (see supplementary methods online). As shown in Fig. 2E, the functional up-regulation of CCR7 was similar in WT and P2Y$_2$R−/− animals.

ATP-induced cytokine production, maturation, and Th2 priming in mDCs is not affected by P2Y$_2$R deficiency

ATP has been shown to modulate cytokine production, maturation, and T-cell priming capacity of human and

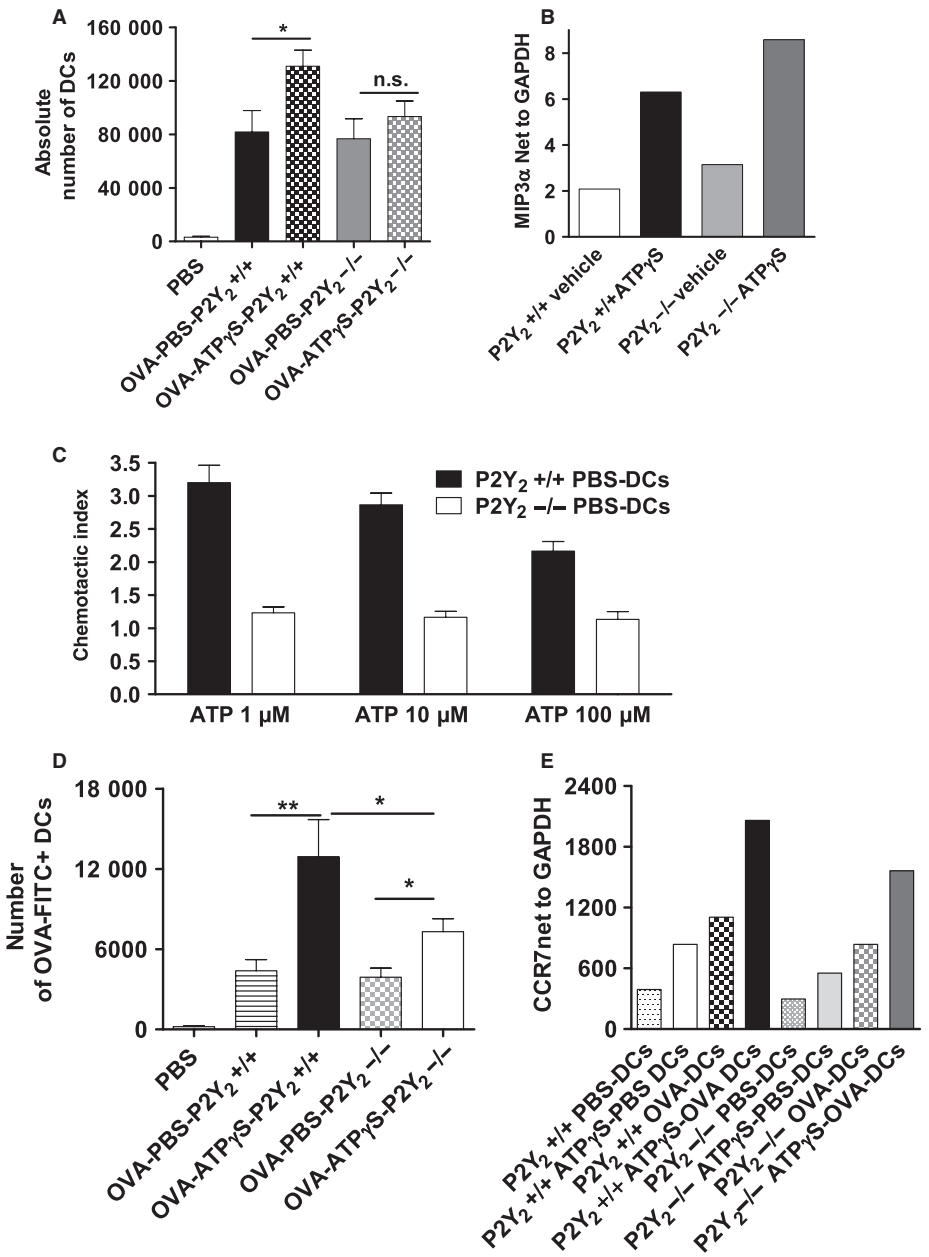

Figure 2 Involvement of P2Y$_2$R in ATP-induced migration of DCs *in vivo* and *in vitro*. (A) Effect of ATPγS on the absolute number of DCs in the lungs of WT and P2Y$_2$R−/− animals. (B) ATPγS exposure to the lungs induces up-regulation of CCL20. (C) ATP fails to induce migration in immature bone marrow–derived dendritic cells (BMDCs) from P2Y$_2$R−/− animals. (D) Migration of DCs to MLN in response to ATPγS is reduced in P2Y$_2$R−/− deficient animals. (E) Influence of ATP on CCR7 mRNA expression by BMDCs. Mean of 6–8mice per group $^*P < 0.05$.

murine mDCs, thereby favouring the outcome of Th2-dominated immune responses (13). We therefore investigated whether these ATP-triggered effects are altered in P2Y$_2$R-deficient DCs. As shown in Fig. 3A, there were no significant differences in ATP-triggered modulation of IL-12p70, TNF-α and IL-10 production in mDCs from OVA-pulsed WT and P2Y$_2$R−/− mDCs mice. In addition, the priming capacity of OVA-pulsed P2Y$_2$R−/− and ATP-stimulated mDCs was not altered compared to WT DCs when co-cultured with OVA-specific T cells obtained from TCR Tg OTII mice (Fig. 3B). Finally, P2Y$_2$R deficiency did not alter ATP-induced up-regulation of CD40, CD80, CD83, and CD86 in OVA-pulsed mDCs (data not shown).

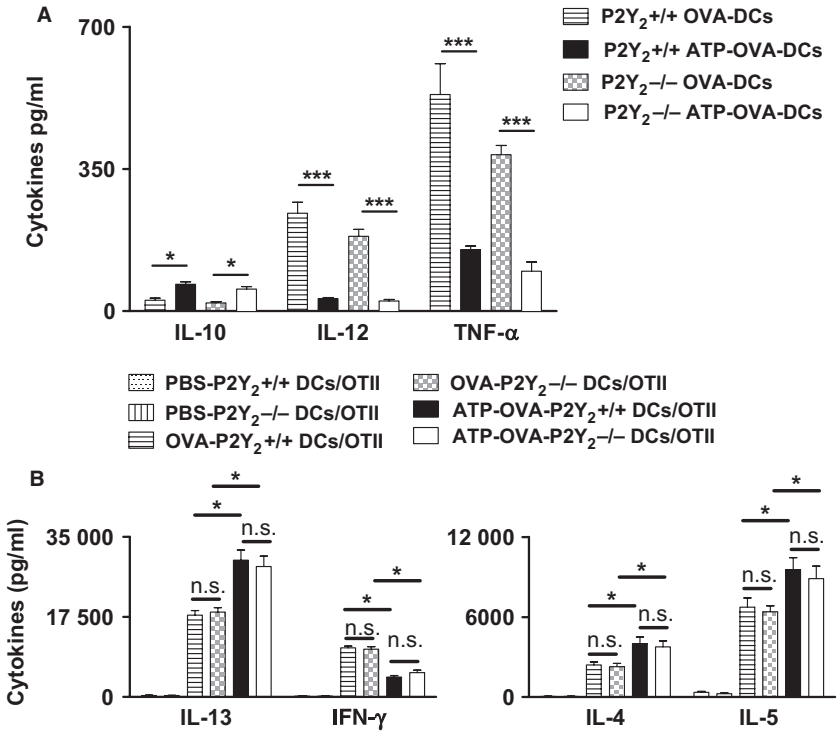

Figure 3 Effect of ATP on cytokine production and T-cell priming capacity of bone marrow–derived dendritic cells (BMDCs) generated from P2Y$_2$R–/– and WT animals. (A) WT and P2Y$_2$R–/– BMDCs were stimulated with ATP ± OVA. Cytokine levels in the supernatant were measured 24 h later. (B) WT and P2Y$_2$R–/– BMDCs were exposed to ATP ± OVA were co-cultured with purified naïve OVA-specific CD4$^+$ T cells. Four days later, IL-4, IL-5, IL-13, and IFN-γ concentrations in supernatants were analysed. Means ± SEM. One representative result out of four is shown.

Der p1 up-regulates P2Y$_2$R expression in human DCs from HDM-sensitized individuals

To test the relevance of these findings for human allergic disorders we investigated the role of the P2Y$_2$R in ATP-induced migration of human monocyte-derived DCs. For this purpose we tested the functional expression of P2Y$_2$R in human monocyte-derived DCs obtained from the peripheral blood of asthmatics allergic to house dust mite following exposure to the major house dust mite allergen Der p1. As shown in Fig. 4, pulsing mDCs from house dust mite allergics with Der p1 (endotoxin low) resulted in an up-regulation of the P2Y$_2$R. This was not observed with DCs obtained from healthy controls (data not shown). The Der p1–induced up-regulation of P2Y$_2$R was associated with increased chemotaxis in response to ATP (Fig. 4B). To exclude that the effects elicited by Der p1 were because of the contamination with endotoxin, cells were also stimulated with LPS. As described previously, the migratory capacity towards ATP was lost following pulsing of DCs with LPS (data not shown) (15).

Effect of ATP on the function of eosinophils

As eosinophils are among the key effector cells in allergic asthma, we also assessed the functional expression of P2Y$_2$R in human blood eosinophils. P2Y$_2$R expression was up-regulated on eosinophils from patients with allergic asthma compared to healthy controls following stimulation with Der p1 (Fig. 5A). Again this was accompanied by a stronger chemotactic response (Fig. 5B).

In addition, the P2Y$_2$R-linked production of reactive oxygen species (ROS) by eosinophils was measured in Ca^{2+}-free medium to exclude the involvement of P2XR subtypes (19). As shown in Fig. 5C, this was also increased in patients with asthma compared to controls. To support the involvement of P2Y$_2$ receptors, cells were also stimulated with UTP, which preferably binds to P2Y$_2$R and has no activity on P2XR-subtypes. Again, eosinophils from asthmatic individuals were more sensitive to the UTP-induced migration and ROS production (Fig. 5C,D). Finally, ATP was not able to induce migration of eosinophils generated from bone marrow of P2Y$_2$R−/− animals (Fig 5E).

Discussion

In this study we demonstrate that allergic lung inflammation is decreased in animals lacking the P2Y$_2$ receptor. Functional studies revealed that this reduction can be explained by the impaired response of dendritic cells and eosinophils to ATP-triggered chemotaxis. Recently, ATP, which is a potent agonist at the P2Y$_2$ receptor (10), has

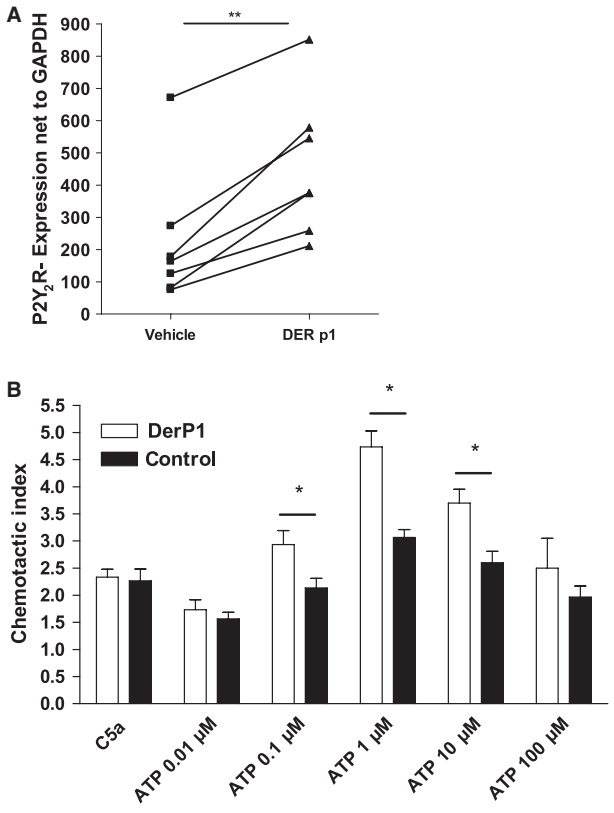

Figure 4 Der p1 induces functional up-regulation of P2Y$_2$R on human monocyte-derived DCs generated from the blood of HDM-allergic asthmatics. (A) DCs generated from the blood of HDM-allergic patients were pulsed with Der p1 or vehicle overnight. P2Y$_2$ receptor expression was analysed by quantitative RT-PCR mean ± SEM $n = 6$. $P < 0.0156$ (Mann–Whitney test) (B) Migration in response to ATP was compared using the modified transwell chamber. Mean ± SEM $n = 6$. $^*P < 0.05$ (Mann–Whitney test).

been found to play a role in the initiation and maintenance of asthmatic airway inflammation, via the recruitment and activation of dendritic cells (13). Additionally, compounds affecting dendritic cell migration, e.g. prostacyclin, FTY720, or broad spectrum antagonists at purinergic receptors, decrease asthmatic airway inflammation (13, 20, 21). ATP is a potent chemoattractant for human and murine dendritic cells (13, 15). However, the exact receptor subtype mediating this response has not yet been identified. In this study, P2Y$_2$R−/− animals did not recruit DCs to the lungs following i.t. application of ATP analogue ATPγS. Previously we reported that ATPγS up-regulates lung CCL20 expression, a chemokine attracting immature blood DCs into the mucosa. Therefore, we speculated that beside the direct ATP-induced chemotaxis an indirect CCL20-triggered migration might be involved in the recruitment of blood DCs to the lungs following ATP-OVA-FITC application (13). However, as the ATP-induced up-regulation of CCL20 was not altered in P2Y$_2$R−/− animals compared to WT controls, *in vitro* experiments clearly showed that BM-derived DCs from P2Y$_2$R−/− animals were unable to migrate in response to ATP; our data suggest the direct involvement of P2Y$_2$R in the recruitment of DCs to the lungs. In P2Y$_2$R−/− animals the number of antigen-laden DCs which reached the MLNs was significantly lower compared to WT animals. Yet, there was a slightly increased number of antigen-laden DCs in ATP-treated compared to vehicle-treated P2Y$_2$R−/− animals which might be attributed to ATP-induced up-regulation CCR7 (22). P2Y$_2$R expression was also up-regulated in human monocyte-derived DCs by house dust mite allergen which resulted in increased migration towards ATP. Consecutively, there is good evidence that the ATP-induced migration of DCs is mediated by P2Y$_2$R in humans and mice. Similar observations have been made with neutrophils whose migratory response to ATP is controlled by P2Y$_2$R and – when metabolized to adenosine – A3 receptors (23, 24). Apart from oriented migration, the activation of purinergic receptors on DCs has been linked to altered surface molecule expression, cytokine secretion, and T-cell priming capacity (13, 25, 26). As this was not affected in DCs derived from P2Y$_2$R-deficient animals, P2Y$_2$ does not seem to be the only purinergic receptor involved in asthma pathophysiology. This is further supported by previous findings where unselective blockade of purinergic receptors, e.g. by suramin or PPADS, leads to a stronger reduction in experimental asthma compared to P2Y$_2$R deficiency alone (13).

Purinergic receptors are also expressed on eosinophils which are among the major effector cells in asthma. ATP has been reported to activate various cell functions such as migration, cytokine, and ROS production in eosinophils (3, 19, 27). The functional expression of P2Y$_2$R in human and murine eosinophils revealed that the migratory response to ATP was also absent in eosinophils derived from P2Y$_2$R-deficient animals. The hypothesis that P2Y$_2$R signalling might contribute to the pathogenesis of asthma is further supported by the observation that eosinophils from asthmatic subjects showed a higher expression of P2Y$_2$R compared to healthy individuals. This appears to be of functional relevance, as these cells were more sensitive to ATP-induced migration and production of reactive oxygen species.

ATP is released into the airways following allergen challenge in humans and mice. Thus, the ligand for P2Y$_2$ receptors to mediate the recruitment of eosinophils into the lungs is present (13). In addition, P2Y$_2$R might play a role in tissue damage via stimulation of ROS release. In conclusion, beside its role in DCs migration, P2Y$_2$R signalling plays a pivotal role in ATP-induced migration and ROS production in human and murine eosinophils.

In summary, our data show that the purinergic receptor subtype P2Y$_2$ is involved in the pathogenesis of experimentally

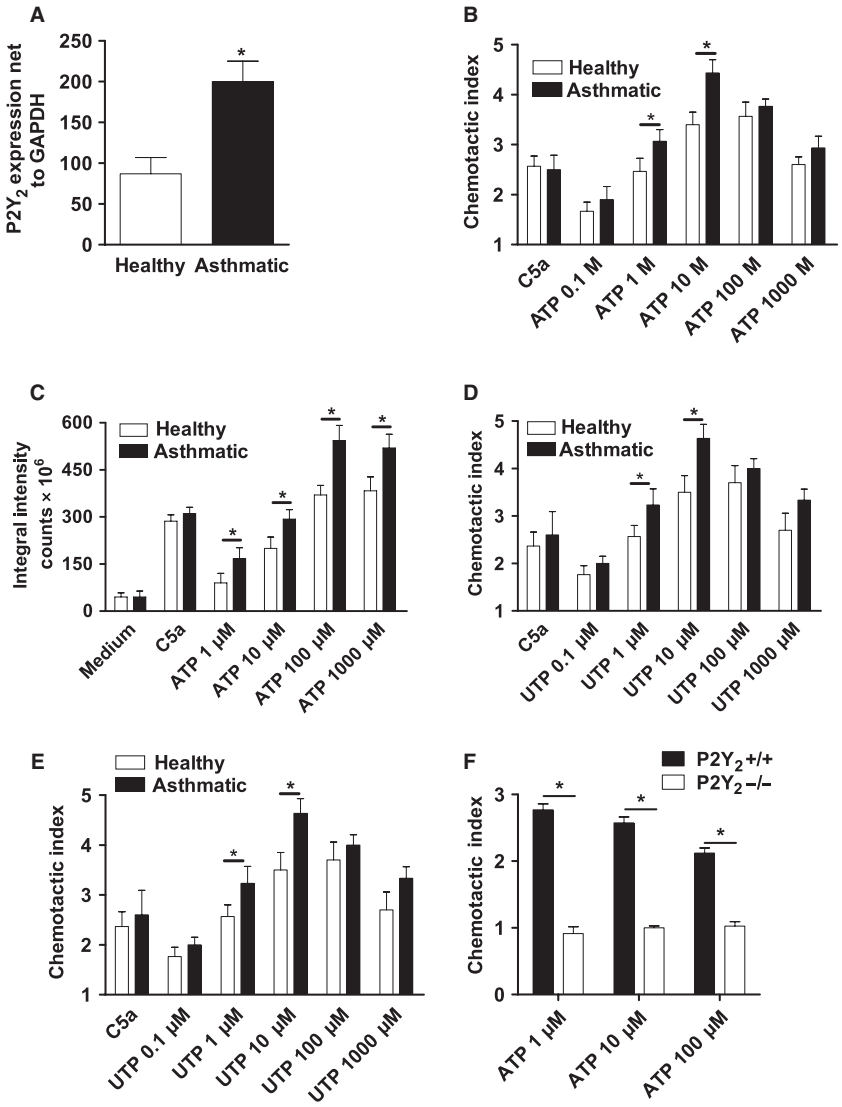

Figure 5 Functional up-regulation of P2Y$_2$ receptors on eosinophils from patients with asthma is associated with enhanced migration and reactive oxygen species (ROS) production *in vitro*. (A) P2Y$_2$ receptor expression by eosinophils from asthmatics ($n = 8$) and healthy controls ($n = 8$) Data are shown as mean ± SEM $n = 8$. $P < 0.002$ (Mann–Whitney test) (B–D) Migration in response to ATP, or UTP or C5a (10^{-7} M) was compared using the transwell chamber system. (C–E) Oxygen radical production in response to ATP, UTP or C5a. Mean ± SEM $n = 8$. *$P < 0.05$ (Mann–Whitney test) (F) ATP fails to induce migration of eosinophils from P2Y$_2$R–/– animals. Mean ± SEM, $n = 3$. *$P < 0.05$ (Mann–Whitney test).

induced allergic lung inflammation via regulating the chemotaxis of dendritic cells and eosinophils to the lung. Therefore, targeting P2Y$_2$ receptors might be a novel approach for the treatment of asthma in humans.

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Supporting Information

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

Data S1. Patient characteristics.

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