

The Journal of Immunology

This information is current as
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J. Immunol. published online Aug 18, 2010;
doi:10.4049/jimmunol.0903908

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P2Y₂ Receptor Regulates VCAM-1 Membrane and Soluble Forms and Eosinophil Accumulation during Lung Inflammation

Gilles Vanderstocken,* Benjamin Bondue,* Michael Horckmans,* Larissa Di Pietrantonio,* Bernard Robaye,[†] Jean-Marie Boeynaems,*[‡] and Didier Communi*

ATP has been defined as a key mediator of asthma. In this study, we evaluated lung inflammation in mice deficient for the P2Y₂ purinergic receptor. We observed that eosinophil accumulation, a distinctive feature of lung allergic inflammation, was defective in OVA-treated P2Y₂-deficient mice compared with OVA-treated wild type animals. Interestingly, the upregulation of VCAM-1 was lower on lung endothelial cells of OVA-treated P2Y₂^{-/-} mice compared with OVA-treated wild type animals. Adhesion assays demonstrated that the action of UTP on leukocyte adhesion through the regulation of endothelial VCAM-1 was abolished in P2Y₂-deficient lung endothelial cells. Additionally, the level of soluble VCAM-1, reported as an inducer of eosinophil chemotaxis, was strongly reduced in the bronchoalveolar lavage fluid (BALF) of P2Y₂-deficient mice. In contrast, we observed comparable infiltration of macrophages and neutrophils in the BALF of LPS-aerosolized P2Y₂^{+/+} and P2Y₂^{-/-} mice. This difference could be related to the much lower level of ATP in the BALF of LPS-treated mice compared with OVA-treated mice. Our data define P2Y₂ as a regulator of membrane and soluble forms of VCAM-1 and eosinophil accumulation during lung inflammation. *The Journal of Immunology*, 2010, 185: 000–000.

Asthma is a Th2 lymphocyte-associated inflammatory airway disease characterized by airway eosinophilia, airway obstruction, and bronchial hyperresponsiveness (1). ATP is now considered an important mediator in this disease (2). High levels of ATP are found in the airways of OVA-sensitized mice after allergen exposure, and asthma features are suppressed using P2R antagonists or ATP-neutralizing apyrase (2).

Infiltration of the airways by eosinophils seems to be central in the pathogenesis of asthma (3–6). It involves the expression of adhesion molecules on the inflamed vascular endothelium. Eosinophils express $\alpha 4 \beta 1$ integrins (VLA-4) that bind to VCAM-1. Mice genetically deficient for VCAM-1 fail to develop pulmonary eosinophilia (3).

P2Y₂R is a ubiquitous G-protein coupled receptor that is fully activated by ATP and UTP (7), and it was described as a target for cystic fibrosis therapy (8). It was shown that P2Y₂ mediates ATP/UTP-induced VCAM-1 upregulation in coronary artery endothelial cells (ECs) (9). This upregulation involves the trans-

activation of vascular endothelial growth factor receptor-2/Flk-1 by P2Y₂R (10).

In this study, we investigated whether P2Y₂ knockout mice display any defect in VCAM-1 expression or in the infiltration of eosinophils and other leukocytes during lung inflammation.

Materials and Methods

Animals

P2Y₂R knockout (P2Y₂^{-/-}) mice were provided as breeder pairs (on a B6D2 genetic background) by Dr. B.H. Koller (University of North Carolina, Chapel Hill, NC). B6D2 P2Y₂^{-/-} mice were then crossed with the SV129 mouse strain by Dr. J. Leipziger (Institute of Physiology and Biophysics, University of Aarhus, Aarhus, Denmark), generating B6D2/SV129 P2Y₂^{+/+} and B6D2/SV129 P2Y₂^{-/-} littermates. Mice were then backcrossed onto C57Bl6 for >10 generations by one of the authors (B.R.). All procedures were reviewed and approved by the local ethical committee (Commission d'Ethique du Bien Etre Animal, Université Libre de Bruxelles).

OVA exposure model

Male mice (6–8 wk old) were exposed to OVA, as previously described (11). Briefly, mice were sensitized with 10 μ g OVA (grade V; Sigma-Aldrich, Bornem, Belgium) adsorbed to 1 mg Al(OH)₃ (Sigma-Aldrich) i.p. on days 0 and 7. From day 14 onward, mice were exposed to aerosolized OVA (1% w/v, grade III; Sigma-Aldrich) or PBS for 30 min using an ultrasonic nebulizer, three times per week for 2 wk. Mice were killed 24 h after the last exposure.

Histological analysis of inflamed lungs of P2Y₂^{+/+} and P2Y₂^{-/-} mice

After fixation of the left lobe of lungs with 4% paraformaldehyde, slices were embedded in paraffin for histology. Sections of 7 μ m were stained with H&E.

LPS-induced acute lung injury model

Pseudomonas aeruginosa endotoxin serotype 10 (Sigma-Aldrich) was prepared as a 10 mg/3 ml saline solution. Male animals (6–8 wk old) were exposed to the aerosolized endotoxin for 20 min. Mice were killed 6 or 24 h after treatment.

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Received for publication December 7, 2009. Accepted for publication July 15, 2010.

This work was supported by an Action of Concerted Research of the French Community of Belgium, Prime Minister's Office, Federal Service for Science, Technology and Culture, by grants from the Fund of Scientific and Medical Research, the Emile DEFAY Fund, the Walloon Region (Programme of Excellence), and the LifeSci-Health programme of the European Community (Grant LSHB-2003-503337). D.C. is a research associate of the National Fund of Scientific Research. G.V., B.B., and M.H. are supported by the National Fund of Scientific Research, Belgium.

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Abbreviations used in this paper: BALF, bronchoalveolar lavage fluid; DC, dendritic cell; EC, endothelial cell; ns, not significant; sVCAM-1, soluble VCAM-1.

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Bronchoalveolar lavage fluid of inflamed P2Y₂^{+/+} and P2Y₂^{-/-} mice

Mice were killed with an i.p. injection of Pentothal (300 mg/kg; Hospira, Brussels, Belgium) and exsanguination. Bronchoalveolar lavage fluid (BALF) was obtained by flushing the lungs with 1 ml sterile 0.9% NaCl. Cytospin preparations were prepared using a Shandon Cytospin III cyto-centrifuge (Thermo Fisher Scientific, Leicestershire, U.K.). Cell counts were performed on cytospin preparations after Diff-Quick staining (Dade Behring, Deerfield, IL) and by flow cytometry.

Flow cytometry analysis of VCAM-1 expression in lung ECs

Lungs were perfused with 10 ml PBS through the right ventricle, dissected, minced, and incubated with 2 mg/ml collagenase D and 0.02 mg/ml DNase I (Roche Diagnostics, Vilvorde, Belgium) for 1 h at 37°C. Cells were stained with CD31-PE (isotype: rat [LEW] IgG2a, κ) and VCAM-1-FITC (isotype: rat IgG2a, κ [LEW]) for flow-cytometry analysis. Flow-cytometry data acquisition was performed on a dual-laser FACSCalibur flow cytometer running CELLQuest software (BD Biosciences, Erembodegem, Belgium). WinMDI software was used for data analysis.

Leukocyte adhesion assay on primary lung ECs

Primary ECs were isolated from P2Y₂^{+/+} and P2Y₂^{-/-} lungs. Briefly, lungs were collected from 6–8-wk-old P2Y₂^{+/+} and P2Y₂^{-/-} mice and minced using sterile razorblades. After treatment with collagenase (2 mg/ml) and DNase I (0.5 mg/ml), primary ECs were removed from the interface of a 25–40% Percoll gradient. Cells were plated on collagen-coated six-well dishes at 5.10⁵ cells per well (BD Biosciences) in complete medium containing endothelial basal medium and endothelial growth medium singlequots (2% FBS, 10 ng/ml epidermal growth factor, 1 μg/ml hydrocortisone, and 12 μg/ml bovine brain extract) from Clonetics (BioWhittaker Europe, Cambrex, Verviers, Belgium) and 100 μg/ml heparin (Sigma-Aldrich). The purity of the cultures was checked using CD31 staining. Cells were stimulated with 100 μM UTP for 24 h in the presence or absence of anti-VCAM-1 blocking

Ab (5 mg/ml) for the last 3 h of nucleotide stimulation. RAW 264.7 macrophages labeled for 2 h with calcein (10 μg/ml) were laid, at 5.10⁵ cells per dish, on confluent P2Y₂^{+/+} and P2Y₂^{-/-} ECs. After 2 h at 37°C, dishes were rinsed twice with PBS; adhesion of RAW264.7 macrophages was measured by manual counting of 15 randomly chosen fields per experimental point using a microscope at ×100 magnification.

Quantification of IgE serum level and soluble VCAM-1 level in BALF of P2Y₂^{+/+} and P2Y₂^{-/-} mice

Total IgE serum level and soluble VCAM-1 (sVCAM-1) BALF levels were measured in P2Y₂^{+/+} and P2Y₂^{-/-} mice using ELISA kits from BD Biosciences and R&D Systems (Abingdon, U.K.), respectively, and following the manufacturers' instructions.

Quantification of leukocyte infiltration by flow-cytometry analysis

Markers used for mouse macrophage staining were anti-F4/80-FITC and anti-CD11b-PE, and markers used for mouse neutrophils included anti-Ly6G-FITC and anti-CD11b-PE (BD Biosciences). Anti-rat (DA) IgG2b (κ-PE), anti-rat IgG2a (κ-FITC), and anti-rat (LEW) IgG2a (κ-FITC) (BD Biosciences) were used as control isotypes for CD11b, F4/80, and Ly6G, respectively. Markers for mouse eosinophil staining were anti-CD49d-FITC and anti-CCR3-PE; anti-rat (F344) IgG2b (κ-FITC) and anti-rat IgG2a (κ-PE) were used as control isotypes, respectively (all from BD Biosciences).

Quantification of ATP level in BALF of LPS- and OVA-treated mice

P2Y₂^{+/+} and P2Y₂^{-/-} mice were exposed to LPS or OVA, as previously described. BALF was collected 24 h after LPS exposure or at the end of the OVA protocol. ATP levels were quantified in the BALF using the luminescence ATP detection assay system ATPlite (PerkinElmer, Zaventem, Belgium).

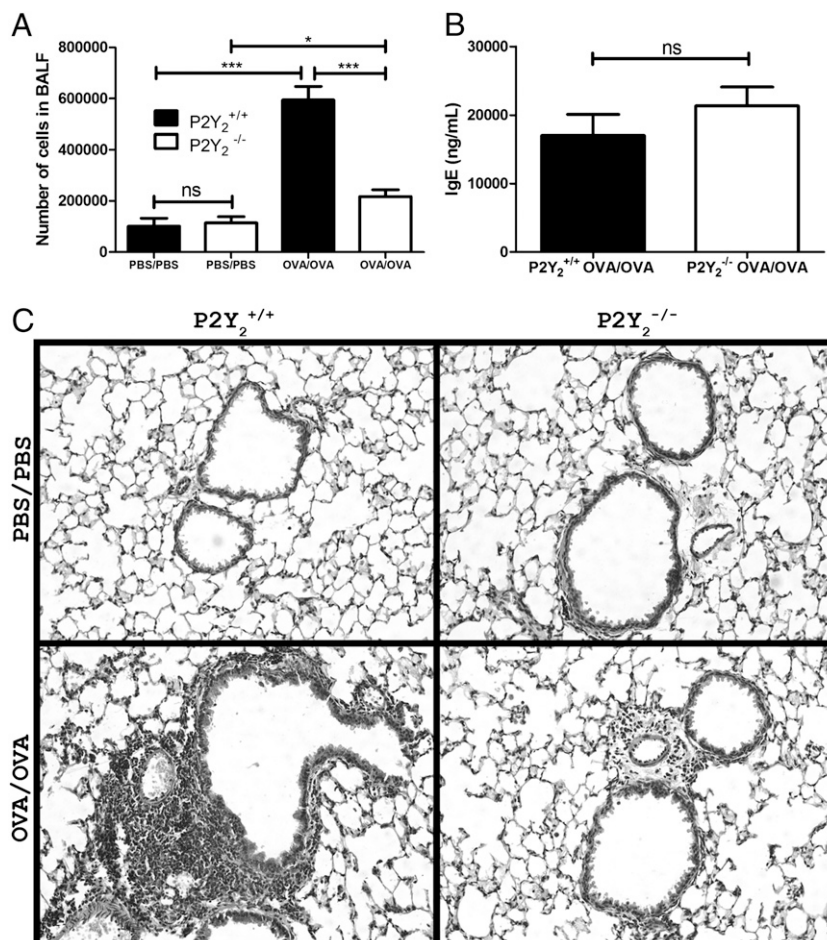


FIGURE 1. Reduced inflammation in the lungs of P2Y₂-deficient asthmatic mice. **A**, Cellular infiltration in the lungs of P2Y₂^{+/+} and P2Y₂^{-/-} control and asthmatic mice. P2Y₂^{+/+} and P2Y₂^{-/-} mice were sensitized and treated with OVA (OVA/OVA) or PBS (PBS/PBS), as described in *Materials and Methods*. Total cell counts were performed in the BALF of asthmatic P2Y₂^{+/+} and P2Y₂^{-/-} mice ($n = 11$). The Student t test was performed using Prism software (GraphPad, San Diego, CA). $*p < 0.05$; $***p < 0.001$. **B**, IgE level in the serum of P2Y₂^{+/+} and P2Y₂^{-/-} asthmatic mice. IgE level was quantified in the serum of P2Y₂^{+/+} and P2Y₂^{-/-} asthmatic mice by ELISA ($n = 11$). **C**, Histological analysis of inflamed lungs of P2Y₂^{+/+} and P2Y₂^{-/-} asthmatic mice. Paraffin sections (7 μm) of lungs of P2Y₂^{+/+} and P2Y₂^{-/-} asthmatic mice were stained with H&E. Original magnification ×20. ns, not significant.

Statistical analysis

Results are presented as mean $\pm$ SEM. For all experiments, the statistical significance between samples was calculated using the Student *t* test. The normal distribution of the data was checked using Kolmogorov–Smirnov, D'Agostino–Pearson, and Shapiro–Wilk tests.

Results

Airway inflammation is defective in OVA-treated $P2Y_2$ -deficient mice

We compared the inflammatory response of $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ mice in a classical asthma model. After PBS or OVA sensitization, $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ mice were aerosolized with PBS (PBS/PBS) or OVA (OVA/OVA), respectively. BALF was recovered from these mice for total cell counting, cytospin, and flow-cytometry analysis. A strong increase in cell number was observed after OVA challenge in $P2Y_2^{+/+}$ mice (Fig. 1A). Interestingly, the total cell number was significantly lower in BALF isolated from $P2Y_2^{-/-}$ mice compared with $P2Y_2^{+/+}$ BALF (72.6 $\pm$ 12.8% of inhibition; ****p* < 0.001; *n* = 11) (Fig. 1A). The sensitization to OVA was comparable in $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ mice, as shown by the measurement of IgE levels (Fig. 1B). H&E coloration of inflamed lungs confirmed the absence of massive leukocyte accumulation in $P2Y_2^{-/-}$ asthmatic mice (Fig. 1C).

Cytospin preparations of BALF were performed to identify leukocyte subpopulations (Fig. 2A). The populations observed in the BALF were eosinophils and macrophages (Fig. 2A). No neutrophils or lymphocytes were detected in our cytospin preparations (data not shown). Interestingly, the strong accumulation of eosinophils observed after OVA treatment of $P2Y_2^{+/+}$ mice was not observed in $P2Y_2^{-/-}$ mice (Fig. 2B). A dramatic reduction in eosinophil number was observed in $P2Y_2^{-/-}$ OVA-treated mice compared with wild type OVA-treated mice (83.2 $\pm$ 18.2% ; ****p* < 0.001; *n* = 7) (Fig. 2B). No increase in macrophage level was observed after OVA treatment of $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ mice using our

protocol (Fig. 2B). Eosinophil levels were also estimated by flow cytometry using CD49d and CCR3 as markers (Fig. 2C). Flow-cytometry experiments confirmed a strong reduction in CD49d⁺ CCR3⁺ cells in the BALF of $P2Y_2^{-/-}$ OVA-treated mice compared with $P2Y_2^{+/+}$ OVA-treated mice (85.1 $\pm$ 9.3% of inhibition; ****p* < 0.001; *n* = 5) (Fig. 2C).

Reduction of VCAM-1⁺ ECs in the lungs of $P2Y_2$ -deficient asthmatic mice

We evaluated the expression of VCAM-1 in lung ECs isolated from $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ OVA-treated mice. Briefly, lungs were digested with collagenase to perform flow-cytometry experiments. CD31/VCAM-1⁺ cells were quantified in these total cell preparations (Fig. 3A). As expected, the number of VCAM-1/CD31⁺ cells was enhanced after OVA challenge in the lungs of $P2Y_2^{+/+}$ mice (Fig. 3A). The number of VCAM-1/CD31⁺ cells was significantly lower in $P2Y_2^{-/-}$ lung cell preparations compared with $P2Y_2^{+/+}$ lung cell preparations (74.1 $\pm$ 26.3% of reduction; **p* < 0.05; *n* = 6) (Fig. 3A).

sVCAM-1 level is reduced in BALF of $P2Y_2^{-/-}$ asthmatic mice

The level of sVCAM-1, previously described as an inducer of eosinophil chemotaxis, was measured in the BALF of PBS- and OVA-treated $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ mice using a specific ELISA (Fig. 3B). We observed that the sVCAM-1 level was strongly increased in $P2Y_2^{+/+}$ mice after OVA treatment (Fig. 3B). Interestingly, there was a significant reduction in the sVCAM-1 level in the BALF of OVA-treated $P2Y_2^{-/-}$ mice compared with OVA-treated $P2Y_2^{+/+}$ mice (59.4 $\pm$ 11.8% of reduction; ****p* < 0.001; *n* = 11) (Fig. 3B).

UTP increased leukocyte adhesion on lung ECs through $P2Y_2$ activation and VCAM-1 increase

We then performed adhesion assays to investigate further a direct link between $P2Y_2$ and VCAM-1. We evaluated the adhesion of leu-

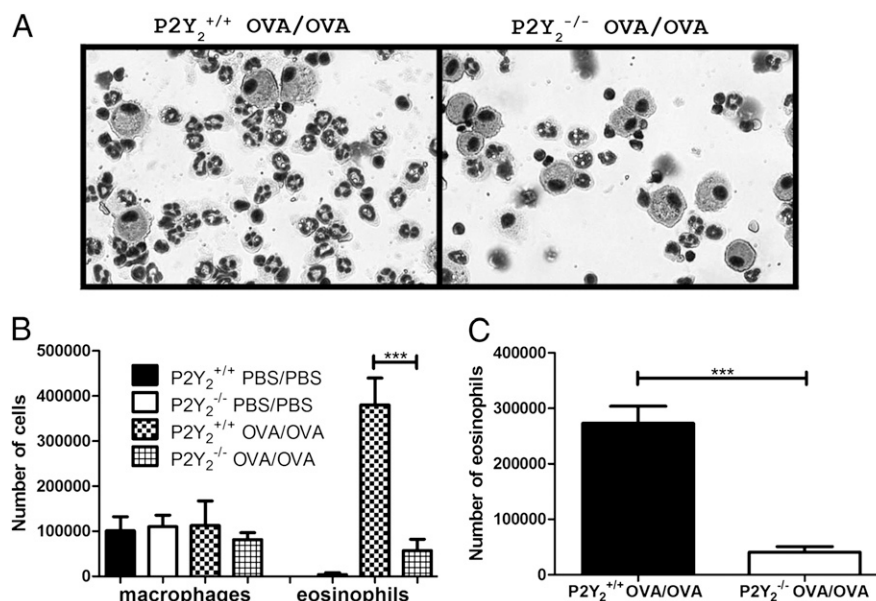


FIGURE 2. Defective eosinophil infiltration in the lungs of $P2Y_2$ -deficient asthmatic mice. **A**, Cytospin preparations of BALF from $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ asthmatic mice. $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ mice were sensitized and treated with OVA (OVA/OVA) or PBS (PBS/PBS), as described in *Materials and Methods*. Cytospin preparations were made using a Shandon III cytocentrifuge and were stained using Diff-Quick staining. Original magnification $\times 40$. **B**, Macrophages and eosinophils were blindly counted in cytospin preparations of BALF from $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ control (PBS/PBS) or asthmatic (OVA/OVA) mice. Results represent the mean $\pm$ SEM of seven randomly selected fields per cytospin preparation (*n* = 7). **C**, Flow-cytometry quantification of CD49d⁺ CCR3⁺ cells in the BALF of $P2Y_2^{+/+}$ and $P2Y_2^{-/-}$ asthmatic mice. Eosinophil quantification in BALF was estimated by flow cytometry using anti-CD49d-FITC and anti-CCR3-PE markers. Flow-cytometry data acquisition was performed on a FACSCalibur flow cytometer. WinMDI software was used for data analysis (*n* = 5). The Student *t* test was performed using Prism software. ****p* < 0.001.

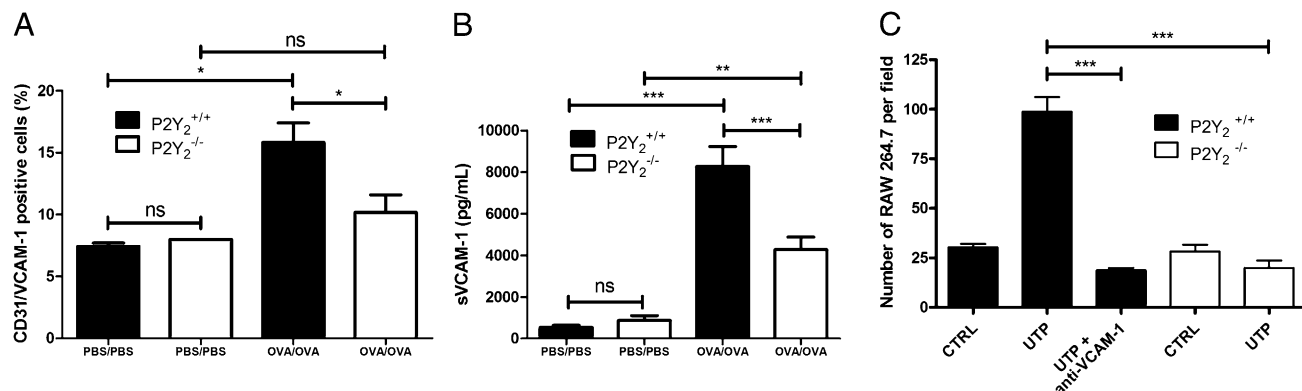


FIGURE 3. Reduced expression of VCAM-1 membrane and soluble forms in the lungs of P2Y₂-deficient asthmatic mice. **A**, Reduction of CD31/VCAM-1⁺ cells in the lungs of P2Y₂^{-/-} asthmatic mice. Lungs of P2Y₂^{+/+} and P2Y₂^{-/-} control (PBS/PBS) or asthmatic (OVA/OVA) mice were digested with collagenase. The number of VCAM-1/CD31⁺ cells was quantified by flow cytometry ($n = 6$). **B**, Reduction of sVCAM-1 level in the BALF of P2Y₂^{-/-} asthmatic mice. sVCAM-1 level was quantified by ELISA in the BALF of P2Y₂^{+/+} and P2Y₂^{-/-} control (PBS/PBS) or asthmatic (OVA/OVA) mice ($n = 11$). **C**, UTP increased macrophage adhesion through P2Y₂ activation and VCAM-1 regulation. Primary ECs were isolated from P2Y₂^{+/+} and P2Y₂^{-/-} lungs and stimulated with 100 μ M UTP in the presence or absence of anti-VCAM-1 blocking Ab ($n = 4$). The Student *t* test was performed using Prism software. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ns, not significant.

kocytes on primary ECs isolated from P2Y₂^{+/+} and P2Y₂^{-/-} lungs. We observed that stimulation of P2Y₂^{+/+} ECs with UTP increased macrophage adhesion (Fig. 3C). This effect was lost when ECs were stimulated with UTP in the presence of anti-VCAM-1 blocking Ab (Fig. 3C). Moreover, there was no effect of UTP on macrophage adhesion using P2Y₂-deficient lung ECs (Fig. 3C).

Comparable recruitment of neutrophils and macrophages in LPS-aerosolized P2Y₂^{+/+} and P2Y₂^{-/-} mice

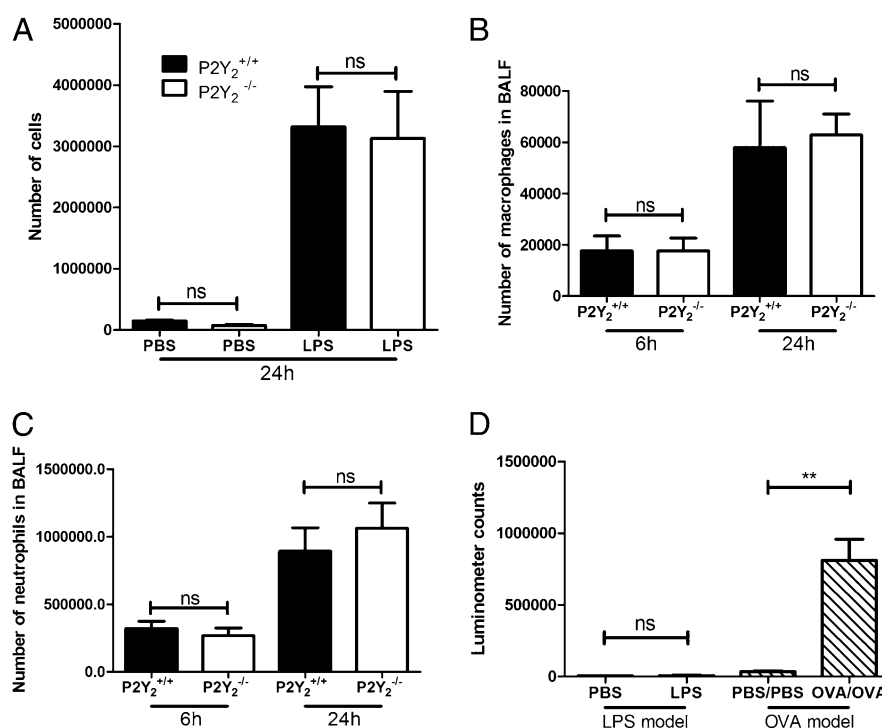
P2Y₂^{+/+} and P2Y₂^{-/-} mice were aerosolized for 20 min with PBS or LPS (3 mg/ml). At 6 or 24 h after LPS exposure, mice were killed, and BALF was collected. LPS induced a massive cellular infiltration in P2Y₂^{+/+} and P2Y₂^{-/-} lungs compared with PBS-treated mice (Fig. 4A). Total cell counting at 24 h was similar in P2Y₂^{+/+} and P2Y₂^{-/-} BALF (Fig. 4A). The recruited leukocyte populations identified in cytospin preparations were neutrophils

and macrophages, and their levels were similar in P2Y₂^{+/+} and P2Y₂^{-/-} BALF (data not shown). Flow-cytometry experiments were performed to quantify macrophages (Fig. 4B) and neutrophils (Fig. 4C). Macrophage and neutrophil levels were similar in the BALF of P2Y₂^{+/+} and P2Y₂^{-/-} mice. We then quantified and compared ATP levels in the BALF of LPS- and OVA-treated mice. ATP levels were clearly much more elevated in the BALF of OVA-treated mice compared with LPS-treated mice (Fig. 4D).

Discussion

ATP was previously defined as a major mediator of lung inflammation by the use of purinergic receptor antagonists or apyrase, which suppress the main features of asthma (2). Allergic asthma is a complex disease characterized by the recruitment of many cell types, such as eosinophils, dendritic cells (DCs), and T lymphocytes, into inflamed lungs. The recruitment and activation of DCs

FIGURE 4. Comparable airway inflammation in LPS-aerosolized P2Y₂^{+/+} and P2Y₂^{-/-} mice. P2Y₂^{+/+} and P2Y₂^{-/-} mice were aerosolized for 20 min with PBS or LPS (3 mg/ml). **A**, BALF was collected, and the total number of cells was evaluated 24 h after LPS exposure. At 6 or 24 h after LPS exposure, flow-cytometry quantification of macrophages (**B**) and neutrophils (**C**) in the BALF was performed. **D**, ATP level was quantified in BALF of LPS- or OVA-treated P2Y₂^{+/+} and P2Y₂^{-/-} mice using the luminescence ATP detection assay system ATPlite. The Student *t* test was performed using Prism software ($n = 7$). ** $p < 0.01$. ns, not significant.



by ATP was proposed to play a major role in asthmatic airway inflammation (2).

To evaluate the possible contribution of P2Y₂R in lung inflammation, inflammatory models involving OVA or LPS administration were performed using P2Y₂^{+/+} and P2Y₂^{-/-} mice. A defective accumulation of eosinophils was observed in P2Y₂-deficient lungs in the OVA asthma model. On the contrary, LPS induced the recruitment of macrophages and neutrophils similarly in P2Y₂^{+/+} and P2Y₂^{-/-} mice. This difference could be explained by a variation in the concentration of ATP in airways; OVA challenge significantly increased the ATP level in BALF, whereas LPS administration had no significant effect.

We decided to investigate the expression of VCAM-1, a major mediator of inflammation, in P2Y₂^{+/+} and P2Y₂^{-/-} asthmatic mice. ATP and UTP are known to regulate the expression of VCAM-1 on coronary artery ECs (9, 10). Endothelial VCAM-1 is a major adhesion molecule for leukocytes, such as macrophages and eosinophils. It is a 100–110-kDa, 715-aa type I transmembrane glycoprotein characterized by the presence of seven C2-type Ig domains (12). VCAM-1 displays an extracellular region of 674 aa, a transmembrane region of 22 aa, and a 19-aa cytoplasmic tail (12). VCAM-1 is expressed by ECs, smooth muscle cells, fibroblasts, macrophages, and neurons. VCAM-1 mediates adhesion of leukocytes expressing α4β1 integrins (VLA-4) in response to inflammatory cytokines and has also been associated with early T cell and B cell maturation (13–15). VCAM-1-deficient embryos were not viable and displayed severe defects in placental and heart development (16). An increase in the level of soluble adhesion molecules has been correlated with a variety of inflammatory diseases (17). The proteolytic cleavage and release of transmembrane surface proteins are important posttranslational mechanisms for the regulation of their functions (18). The ectodomain shedding occurs for proteins, such as growth factors, cytokines, and adhesion molecules, and is mainly mediated by matrix metalloproteinases and disintegrin metalloproteinases. TNF-α-converting enzyme (a disintegrin and metalloproteinase 17) regulates the PMA-induced release of sVCAM-1 by shedding of the VCAM-1 expressed by murine ECs (17).

We decided to evaluate the level of membrane and soluble forms of VCAM-1 in P2Y₂^{+/+} and P2Y₂^{-/-} mice in the asthma model. We observed a significant reduction in VCAM-1 expression on lung ECs of OVA-treated P2Y₂-deficient mice. Moreover, the level of sVCAM-1 was strongly reduced in the BALF of P2Y₂-deficient mice. Interestingly, sVCAM-1 was described recently as an inducer of eosinophil chemotaxis (19). Allergen-induced accumulation of eosinophils was previously associated with increased levels of sVCAM-1 (20). Thus, membrane and soluble forms of VCAM-1 seem to contribute to eosinophil infiltration into inflamed lungs. VCAM-1 hypomorphic mutant mice treated with OVA exhibit severe deficiency in eosinophil infiltration, whereas early macrophage recruitment is normal (3).

In OVA-treated P2Y₂^{-/-} mice, defects in the membrane and soluble forms VCAM-1 were observed concomitantly with a dramatic decrease in eosinophil recruitment. Nevertheless, the ubiquitous expression of P2Y₂R suggests that other inflammatory defects in P2Y₂^{-/-} mice might contribute to the phenotype. P2Y₂Rs are also expressed on the surface of eosinophils and could also be directly involved in their chemotaxis. Furthermore, Idzko et al. (2) described the important contribution of ATP to the activation and migration of lung myeloid DCs. Intrapulmonary application of ATPγS with OVA potentiated a Th2 response, revealing adjuvant properties of ATPγS related to its effect on DC maturation and migration (2). It is interesting to note that the ATP actions on murine DCs are largely mediated by its degradation into adenosine

and the activation of A_{2B} receptors (21). Our adhesion data and quantification of VCAM-1 levels support that the proinflammatory action of ATP in the lung could occur through DC activation, as well as through the regulation of endothelial and sVCAM-1 expression. Moreover, the P2Y₂ receptor also plays a determinant role in the function of lung epithelial cells as a target in cystic fibrosis therapy (8) and as a regulator of their secretion of inflammatory cytokines, such as IL-8 and CCL-20 (22). The complexity of the contribution of P2Y₂ in lung inflammation is likely to result from combined action of nucleotides on ECs, epithelial cells, and leukocytes.

The present study defines P2Y₂R as a regulator of membrane and soluble forms of VCAM-1 mediating the adhesion and migration of eosinophils and as a target of therapeutic drugs reducing asthmatic inflammation.

Disclosures

The authors have no financial conflicts of interest.

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