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Role of the P2Y\textsubscript{12} Receptor in the Modulation of Murine Dendritic Cell Function by ADP

Abdelhakem Ben Addi,*1 Dorothée Cammarata,*,† Pamela B. Conley,* Jean-Marie Boeynaems,‡§ and Bernard Robaye*

The effects of ADP on the biology of dendritic cells have been studied much less than those of ATP or adenosine. In this study, we showed that adenosine-5'-O-(2-thiodiphosphate) (ADP\textsubscript{S}) induced intracellular Ca\textsuperscript{2+} transients in murine dendritic cells (DCs). This effect was abolished by AR-C69931MX, a dual P2Y\textsubscript{12} and P2Y\textsubscript{13} receptor antagonist. RT-PCR experiments revealed the expression of both P2Y\textsubscript{12} and P2Y\textsubscript{13} mRNA in DCs. The Ca\textsuperscript{2+} response to ADP\textsubscript{S} was maintained in P2Y\textsubscript{13}-deficient DCs, whereas it was abolished completely in P2Y\textsubscript{12}−/− DCs. ADP\textsubscript{B}S stimulated FITC-dextran and OVA capture in murine DCs through macrophinocytosis, and this effect was abolished in P2Y\textsubscript{12}−/− DCs. ADP\textsubscript{B}S had a similar effect on FITC-dextran uptake by human monocyte-derived DCs. OVA loading in the presence of ADP\textsubscript{B}S increased the capacity of DCs to stimulate OVA-specific T cells, whereas ADP\textsubscript{S} had no effect on the ability of DCs to stimulate allogeneic T cells. Moreover, after immunization against OVA, the serum level of anti-OVA IgG1 was significantly lower in P2Y\textsubscript{12}−/− mice than that in wild-type controls. In conclusion, we have shown that the P2Y\textsubscript{12} receptor is expressed in murine DCs and that its activation increased Ag endocytosis by DCs with subsequent enhancement of specific T cell activation. 


Dendritic cells (DCs) are the most potent APCs of the immune system (1, 2). These cells are distributed widely in the body and regulate both immunity and immune tolerance. Within peripheral tissues, immature DCs collect antigenic material and then traffic to secondary lymphoid organs, where they communicate, as mature DCs, with lymphocytes to orchestrate adaptive immune responses.

The effects of extracellular ATP and adenosine on DC function have been studied extensively. Extracellular ATP induces a semimatured of human monocyte-derived DCs, characterized by increased expression of costimulatory molecules, inhibition of IL-12, and stimulation of IL-10 (3–6). These effects are likely to be mediated by the P2Y\textsubscript{11} receptor (5, 7). Adenosine is well known to exert an anti-inflammatory effect on DCs by activating the A\textsubscript{2A} receptor in murine bone marrow-derived DCs (BMDCs) (8, 9) and the A\textsubscript{3} receptor in human monocyte-derived DCs (10, 11). The A\textsubscript{3} receptor also is expressed on these last cells (12). Adenosine also induces the chemotaxis of immature human plasmacytoid DCs via the A\textsubscript{1} receptor, whereas the A\textsubscript{2A} receptor is upregulated after maturation and inhibits cytokine production (13).

On the contrary, the effects of ADP on DC function have been little investigated. ADP has been shown to trigger a pertussis toxin-sensitive intracellular Ca\textsuperscript{2+} transient in immature human DCs and to activate the MAPK ERK-1 pathway (14, 15). The latter effect was inhibited by AR-C69931MX, a dual P2Y\textsubscript{12} and P2Y\textsubscript{13} receptor antagonist. In addition, ADP inhibits the production of both IL-12 and IL-10 (15), whereas it stimulates the expression of IL-23 p19 subunit mRNA (16). ADP also was reported to be chemotactic for immature DCs, an effect inhibited by pertussis toxin (14).

Taken together, these results suggested the functional expression of G\textsubscript{i/o} protein-coupled ADP receptor(s) on DCs, presumably P2Y\textsubscript{12} or P2Y\textsubscript{13}. Therefore, the aim of the present work was to investigate the effects of ADP on murine DC function and to identify the receptor(s) involved thanks to the use of knockout mice.

Materials and Methods

Reagents

Adenosine-5'-O-(2-thiodiphosphate) (ADP\textsubscript{S}), MRS-2179, (+)-diphenyl-4-[phenylsulfinyl]ethenyl]-3,5-pyrazolidinedione, 3-amino-5-(aminominoethyl)-5-(dimethylamino)-6-chloropyrazinecarboxamide hydrochloride (DAMA), BAPTA-AM, ionomycin, OVA, and FITC-dextran (40 kDa) were purchased from Sigma-Aldrich (St. Louis, MO). AR-C69931MX and AR-C67085MX were gifts from AstraZeneca (London, U.K.), dye-quenched OVA (DQ OVA) plasmonic acid, and Fluoro-4AM were purchased from Invitrogen (Carlsbad, NM).

Animals

P2Y\textsubscript{12}−/− mice were generated as described by Andre et al. (17). They were on a C57BL/6 genetic background. The generation of P2Y\textsubscript{13}−/− mice will be described elsewhere (A.C. Fabre, C. Malaval, A. Ben Addi, C. Verdier, V. Pons, N. Serhan, L. Lichtenstein, G. Combes, N. Nijstad, U. Tietge, et al., manuscript in preparation). In brief, the P2Y\textsubscript{13} gene was disrupted in 129Sv/R1 embryonic stem cell lines after homologous recombination of the targeting vector. P2Y\textsubscript{13}-targeted embryonic stem cells were identified by Southern blot analyses and used for chimeric mice generation by aggregation.
with morulae. Mice with the targeted mutation were crossed with C57BL/6 mice for up to 10 generations. OT-1 and OT-2 Rag−/− mice were provided kindly by Dr. F. Bureau (Université de Liège, Liège, Belgium). The transgenic TCR of OT-1 and OT-2 mice was designed to recognize OVA residues (323–339 and 257–264) in the context of H2Kb and I-Ab, respectively. All of the mice used in this study were housed under specific pathogen-free conditions in our animal facility. All of the animal studies were authorized by the Animal Care Use and Review Committee of the Université Libre de Bruxelles.

DC generation

Splenic DCs (sDCs) were expanded in vivo by s.c. injection of 1 × 10^6 recombinant B16 melanoma cells expressing the murine FLT3 ligand as described previously by Pasare et al. (18). sDCs were purified on MACS cell separation columns using anti-CD11c microbeads (Miltenyi Biotec, Gladbach, Germany). Purity was analyzed by flow cytometry and was consistent with at least 95% CD11c+ cells. BMDCs were generated as described previously by Inaba et al. (19). Briefly bone marrow cells obtained from femur and tibia were cultured for 9 d in complete medium (see below) supplemented with 20 ng/ml GM-CSF (Invitrogen). sDCs and BMDCs were cultured in complete medium: RPMI 1640 supplemented with 5% FCS (MultiCell Technologies, Lincoln, RI), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME. Human monocyte-derived DCs were generated as described previously (5). Briefly, PBMCs were isolated from leukocyte-enriched buffy coats by standard density gradient centrifugation on Lymphoprep solution from Nycomed (Oslo, Norway) and resuspended in complete medium. Cells were allowed to adhere for 2 h at 37˚C on culture plates. Adherent cells were cultured further for 6 d in complete medium supplemented with 800 U/ml GM-CSF and 1000 U/ml IL-4. GM-CSF and IL-4 were added every 3 d.

Isolation of RNA, reverse transcription, and quantitative PCR

Total RNA was extracted using TriPure reagent (Roche, Basel, Switzerland) and quantified on a NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA). After DNase I treatment (Fermentas, Hanover, Germany), RNA was reverse-transcribed using the RevertAid H Minus M-MuLV reverse transcriptase (Fermentas). PCR was performed using the Qiagen Taq Core Kit (QiaGen, Venlo, Netherlands). Quantitative RT-PCR was performed with the quantitative RT-PCR (qPCR) MasterMix for SYBR Green I (Eurogentec, Sint-Pieters-Woluwe, Belgium). All of the primers (Table I) were designed using the Primer3 software (http://fokker.wi.mit.edu/primer3/primer3_code.html). For qPCR, housekeeping genes (CANX, RPL32, and YWHAZ; Biogazelle, Gent, Belgium) were selected using Genorm program.

[Ca2+]i measurement

[Ca2+]i was measured in sDCs and BMDCs as described previously (8). Briefly, freshly isolated sDCs (or BMDCs) were labeled with the calcium fluorescent dye Fluo-4-AM (5 μM) in the presence of 250 μM (±)-diphenyl-4-[phenoxy]methyl)-3,5-pyrazolidinedione and 100 μM fura-2 AM. Cell fluorescence was monitored for 204 s at 37˚C, using a FACScan flow cytometer (BD Biosciences, Franklin Lake, NJ). Antagonists were preincubated for 10 min prior to stimulation. After three washes with cold PBS (supplemented with 0.1% BSA and 0.1% sodium azide), cells were analyzed by flow cytometry (20,000 events per sample were analyzed). In all of the experiments, cells incubated with the probes at 4˚C were used as background controls. Trypan blue (0.05%) was used to quench extracellular fluorescence.

Cytokine measurement

Fresherly isolated sDCs were cocultured for 24 h in complete medium (see above) with 2 × 10^5 irradiated (10,000 rad) 3T3 or 3T3 cells expressing CD40L, kindly provided by Dr. Moser (Université Libre de Bruxelles, Gosselies, Belgium). Cell-free supernatants were harvested, and IL-12p70 levels were determined by a commercially available ELISA kit (eBioscience, San Diego, CA).

Mixed lymphocyte reaction

OT-1, OT-2, and BALB/c T lymphocytes were purified from spleen on MACS cell separation columns by depletion of non-T cells. Purity was analyzed by flow cytometry and was consistent with at least 95% CD3+ cells. OVA-loaded DCs and T cells were cocultured for 72 h, and proliferation of T cells was determined by [3H]thymidine (1 μCi/ml) incorporation in the last 12–16 h of culture.

Mice immunization

Mice were injected s.c. at the base of the tail with an emulsion of IFA and OVA (100 μg per mouse) or with IFA only (non-immunized mice) and bled 16 d later. Serum was obtained after centrifugation of clotted blood (2–6 h at 4˚C). Specific anti-OVA IgGs and total IgG subclass G1 and G2a were quantified by ELISA. Briefly, 96-well flat-bottom plates were coated overnight at 4˚C with OVA (5 μg/ml in PBS) or monoclonal rat anti-mouse IgG1 or IgG2a Abs (5 μg/ml in PBS) to quantitate OVA-specific or total IgG, respectively. Plates were saturated with PBS/0.5% BSA for 1 h at room temperature. Serum samples were diluted in PBS/0.1% BSA and incubated for 2 h at room temperature. Specific and total IgG subclass were detected using HRP-conjugated rat anti-mouse IgG1 or IgG2a Abs (LO-IMEX, Brussels, Belgium).

Statistical analysis

Statistical analyses were performed using Prism, version 3.00, for Windows (GraphPad, San Diego, CA).

Results

Expression of ADP receptor(s) in murine DCs

The functional expression of ADP receptors was investigated in FLT3 ligand expanded murine sDCs and BMDCs. In these experiments, ADPβs was used instead of ADP to minimize effects due to its degradation product adenosine. As shown in Fig. 1A, ADPβs triggered a pertussis toxin-sensitive transient elevation of [Ca2+]i in BMDCs as well as in sDCs. This rise in [Ca2+]i was concentration-dependent: EC50 = 1.9 μM (Fig. 1B). To identify the receptor(s) involved, we used selective antagonists of ADP receptors. MRS-2179, a P2Y1 receptor-selective antagonist, had no effect, whereas AR-C69931MX, a dual P2Y12 and P2Y13 receptor antagonist, completely abolished the [Ca2+]i rise induced by ADPβs in sDCs (Fig. 1C). Furthermore, AR-C67085MX, an antagonist more potent on the P2Y12 receptor than on the P2Y13 receptor (20), also abolished the rise in [Ca2+]i induced by ADPβs. RT-PCR experiments revealed the expression of both P2Y12 and P2Y13 mRNA in sDCs (Fig. 1D) and BMDCs (data not shown). To distinguish the contribution of each receptor, P2Y12− and P2Y13−deficient DCs were used.

Table I. List of specific primers used in this study

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<td>TCCCGGAGACACTCTATATCC</td>
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<td>P2Y13</td>
<td>ATTCGTGGGTGAGGTAGTAA</td>
<td>ATTCGTGGGTGAGGTAGTAA</td>
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</table>
As shown in Fig. 2A, the Ca\textsuperscript{2+} response to ADP\textsubscript{BS} was maintained in P2Y\textsubscript{13}−/− sDCs, whereas it was abolished completely in P2Y\textsubscript{12}−/− sDCs. Surprisingly, the rise in [Ca\textsuperscript{2+}]\textsubscript{i} in P2Y\textsubscript{13}−/− sDCs was larger than that in wild-type cells. To find out the mechanism underlying this alteration, we compared the expression levels of P2Y\textsubscript{12} transcripts in P2Y\textsubscript{13}−/− sDCs and wild-type cells (Table I). As shown in Fig. 2B, the P2Y\textsubscript{12} mRNA level was 2-fold higher in P2Y\textsubscript{13}−/− sDCs than that in wild-type cells. The rise of [Ca\textsuperscript{2+}]\textsubscript{i} also was maintained in P2Y\textsubscript{13}−/− BMDCs (data not shown), whereas it was decreased partially in P2Y\textsubscript{12}−/− BMDCs (Fig. 2C). The remaining calcium response in BMDCs was abolished by MRS-2179, indicating the functional expression of the P2Y\textsubscript{1} receptor in these cells.

FIGURE 1. Expression of ADP receptors in murine DCs. A–C, [Ca\textsuperscript{2+}]\textsubscript{i} measurement in DCs. Cells were loaded with Fluo-4-AM, and fluorescence was monitored as described in Materials and Methods. A, Freshly isolated sDCs (top) or BMDCs (middle and bottom) were stimulated (arrows) with ADP\textsubscript{BS} (10 μM) and then with ionomycin (iono, 300 nM) to control variation of loading. BMDCs (bottom) were preincubated with pertussis toxin (100 ng/ml) during the last 24 h of culture. Data are expressed as mean fluorescence intensities (MFIs). B, sDCs were stimulated with the indicated concentrations of ADP\textsubscript{BS}. Data are expressed as ratios of the maximal response induced by ionomycin. C, sDCs were preincubated with MRS-2179 (MRS, 10 μM), AR-C69931MX (AR-C69, 10 μM), or AR-C67085 (AR-C67, 10 μM) for 10 min and then stimulated with ADP\textsubscript{BS} (10 μM). ***p < 0.001 using Bonferroni’s multiple comparison test. D, RNA of sDCs was extracted as described in Materials and Methods, and RT-PCR was performed using specific primers for P2Y\textsubscript{12} and P2Y\textsubscript{13} cDNA. Negative controls (bottom) of the PCR reactions (RNA without reverse transcription) are shown for each pair of primers. A–C, One representative experiment out of three to five is shown.

Effects of ADP on DC function
After having demonstrated that murine DCs express at least one functional ADP receptor, we studied the effects of ADP\textsubscript{BS} on DC function. First of all, we observed that the IL-12p70 production by CD40L-stimulated sDCs was reduced partially by ADP\textsubscript{BS} (mean inhibition 40 ± 4%; Fig. 3). This partial inhibition was maintained in both P2Y\textsubscript{12}−/− and P2Y\textsubscript{13}−/− sDCs (40 ± 6 and 35 ± 7% inhibition, respectively). Furthermore, MRS-2179 did not abolish the inhibitory effect of ADP\textsubscript{BS} on IL-12 production, excluding the involvement of the P2Y\textsubscript{1} receptor. The inhibition of IL-12 production may be due to the activation of A\textsubscript{2B} receptor by adenosine contaminating or generated from ADP\textsubscript{BS}, as indicated by our previous results (8).

FIGURE 2. Functional expression of the P2Y\textsubscript{12} receptor in mouse DCs. A, [Ca\textsuperscript{2+}]\textsubscript{i} measurement in transgenic sDCs and BMDCs. A, Fluo-4-AM-loaded wild-type (WT), P2Y\textsubscript{13}−/−, and P2Y\textsubscript{12}−/− sDCs were stimulated with 10 μM ADP\textsubscript{BS}. Data are expressed as mean ± SEM of five independent experiments. B, Quantitative RT-PCR (SYBR Green) for P2Y\textsubscript{12} mRNA in WT and P2Y\textsubscript{13}−/− sDCs. C, WT and P2Y\textsubscript{12}−/− BMDCs were preincubated with MRS-2179 (MRS, 10 μM) for 10 min and then stimulated with ADP\textsubscript{BS} (10 μM). Data are expressed as mean ± SEM of three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 using Bonferroni’s multiple comparison test.
DCs have the ability to take up Ags (endocytosis). Depending on the nature of the particle to be internalized, distinct endocytosis mechanisms are involved: phagocytosis, receptor-mediated endocytosis, or macropinocytosis (21). To characterize the effect of ADP on endocytosis, we used FITC-dextran as a fluorescent Ag. As shown in Fig. 4A, ADPβS stimulated FITC-dextran internalization. This effect was concentration-dependent (EC50 = 25.7 μM; Fig. 4B) and was maximal after 30 min of stimulation (Fig. 4C). The stimulatory effect of ADPβS was maintained in P2Y13−/− sDCs (data not shown), whereas it was abolished completely in P2Y12−/− sDCs (Fig. 4D). To identify the mechanism of FITC-dextran uptake induced by ADPβS, we used different inhibitors. As shown in Fig. 4E, DMA, an inhibitor of macropinocytosis (22), abolished the stimulatory effect of ADPβS on FITC-dextran internalization. Furthermore, BAPTA-AM, an intracellular calcium chelator, slightly decreased the basal FITC-dextran internalization, as reported previously by Falcone et al. (23), but blocked completely the stimulatory effect of ADPβS on Ag uptake. In addition, ADPβS (100 μM) increased the uptake of FITC-dextran by human monocyte-derived DCs to 136% of the control (mean of six experiments), whereas ATP (100μM) increased it to 144%, an effect similar to that previously reported in the literature (4).

Because Ag internalization was enhanced by ADPβS, we assessed whether this nucleotide could modulate Ag presentation by DCs. OVA was used, instead of FITC-dextran, due to the availability of transgenic OVA-specific T cells that allow the study of Ag presentation in vitro. OVA is internalized mainly through the mannose receptor but also is taken up by macropinocytosis (24). Because ADP stimulated macropinocytosis, we first investigated the effect of ADPβS on OVA internalization. As shown in Fig. 5A, ADPβS slightly, but significantly, increased OVA uptake by sDCs, and this effect also was abolished completely in P2Y12−/− sDCs. We then cocultured OVA preloaded splenic DCs with OVA-specific T cells. sDCs loaded in presence of ADPβS showed an increased ability to activate OVA-specific T cells (OT-1 and OT-2) (Fig. 5B, 5C). On the contrary, ADPβS did not enhance the capacity of sDCs to activate allogeneic T cells (purified from BALB/c mice) (Fig. 5D), indicating that the stimulatory effect of ADPβS is related mainly to Ag presentation.

Finally, we assessed whether the P2Y12 receptor may modulate an immune response to soluble Ags in vivo. For that purpose, we immunized wild-type and P2Y12−/− mice with OVA and quantified their production of anti-OVA IgS. To enhance the immune response, OVA was injected s.c. as an emulsion in IFA. As shown in Fig. 6, P2Y12−/− mice exhibit a lowered IgG1 response. IgG2a production also was reduced in these knockout mice, but the reduction was not statistically significant. Total IgG1 and IgG2a levels were similar in wild-type and P2Y12−/− mice.

**Discussion**

The P2Y12 receptor has restricted expression. It is known to be expressed on platelets and to play a major role in their aggregation (17, 25). Its presence in numerous regions of the brain is consistent with a glial expression pattern (25). In particular, it is localized on microglial cells and involved in microglial activation (polarization, process extension, and migration) by nucleotides after brain damage (26, 27). Hollopeter et al. (25) showed that the P2Y12 mRNA is not present in peripheral blood leukocytes. However, others have reported that the P2Y12 transcript is expressed in murine...
Langherans cells (28) and lymphocytes (29). Our present results show for the first time the functional expression of the P2Y12 receptor in murine DCs.

We show in the present work that the activation of the P2Y12 receptor increased macropinocytosis by murine DCs, which subsequently increased their ability to stimulate Ag-specific T cells. Similarly, ATP has been shown to transiently stimulate FITC-dextran internalization by human DCs through an unidentified receptor (4). We now have shown that ADPβS exerts a similar effect on human DCs. Our results are also consistent with the fact that other Gi/o protein-coupled receptors are known to stimulate Ag uptake by DCs. Activation of CCR5 and CCR7, two Gi/o-coupled chemokine receptors involved in DC migration, enhanced the endocytotic activity of murine DCs (30).

FIGURE 5. Effect of ADPβS on the Ag presentation ability of DCs. A, Endocytosis of OVA by freshly isolated WT and P2Y12−/− sDCs. Cells were stimulated with ADPβS (50 μM) and simultaneously incubated with DQ OVA (1 mg/ml) for 30 min. Data are expressed as MFI ± SD and are representative of at least three independent experiments. B–D, sDCs were cocultured with T cells purified from OT-2 Rag−/− mice (B), OT-1 Rag−/− mice (C), or BALB/c mice (D) for 72 h, and proliferation was determined by [3H]thymidine incorporation for the last 12–16 h of culture. sDCs were preloaded with OVA (100 μg/ml) and simultaneously stimulated with ADPβS (50 μM) for 1 h. A, ***p < 0.001 using Bonferroni’s multiple comparison test. B–D, ***p < 0.001; **p < 0.01 using Student t test.

FIGURE 6. Ig production in WT versus P2Y12−/− mice. Mice were immunized s.c. with an emulsion of IFA and OVA (100 μg per mouse). Sixteen days after immunization, mice were bled. Specific anti-OVA IgG1 (A) and IgG2a (B) as well as total IgG1 (C) and IgG2a (D) were determined in serum by ELISA. Data are expressed as mean of OD ± SEM from four independent experiments. *p < 0.05 using unpaired t test with Welch’s correction (WT versus P2Y12 knockout are compared at the same dilution). n, number of mice; NI, nonimmunized mice; n.s., not significant.
Treatment with clopidogrel, a selective P2Y12 antagonist, is associated with a marked reduction of inflammatory biomarkers (31–35). However, this anti-inflammatory action is likely to be an indirect effect involving inhibition of platelets that are known to play a key role in vascular inflammation through the expression and release of several proinflammatory mediators (CD40L and P-selectin) (36–38). But our results suggest that the P2Y12 receptor also may directly modulate the immune responses by stimulating Ag endocytosis and presentation by DCs. Because platelets also can enhance Ag presentation, improve CD8+ T cell responses, and play a critical function in normal T-dependent humoral immunity (39), the relative contributions of platelet and DC P2Y12 receptor in the control of immune responses remain to be clarified. Indeed, Paruchuri et al. (40) recently reported that leukotriene E4-induced pulmonary inflammation in OVA-sensitized mice was abolished by clopidogrel and in P2Y12−/− mice, but this was explained entirely by decreased platelet activation, because platelet depletion produced the same effect.

Previous studies have shown that extracellular nucleotides may behave as immunomodulators. Granstein et al. (28) showed that intradermal ATPγS enhanced contact hypersensitivity and the response to a tumor vaccine via the induction of I-κB, CD80, CD86, IL-1β, and IL-12 and stimulation of the Ag-presenting function of Langerhans cells. Furthermore, Idzko et al. (41) showed that ATP enhances in vivo the Th2 sensitization to inhaled Ag in a model of experimentally induced asthma. The adjuvant effects of ATP were attributed to the recruitment and activation of lung myeloid DCs that induced Th2 responses in the mediastinal nodes. In both reports, the receptor mediating the effects of ATP was not identified. One cannot exclude the possibility that at least part of these effects is due to the hydrolysis of ATP into ADP and the activation of the P2Y12 receptor. This is supported by our observation that leukotriene E4-induced pulmonary inflammation in OVA-sensitized mice was abolished by clopidogrel and in P2Y12−/− mice, but this was explained entirely by decreased platelet activation, because platelet depletion produced the same effect.

In conclusion, the current study demonstrates that the P2Y12 receptor is expressed on DCs and stimulates their endocytotic activity and Ag-presenting function.

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Disclosures
The authors have no financial conflicts of interest.

References


