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Human monocyte derived dendritic cells express functional P2X and P2Y receptors as well as ecto-nucleotidases

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Abstract We investigated the expression and function of P2 receptors and ecto-nucleotidases on human monocyte derived dendritic cells (DC). In addition we analyzed the effect of extracellular ATP on the maturation of DC. By RT-PCR, DC were found to express mRNA for several P2X (P2X1, P2X4, P2X5, P2X7) and P2Y (P2Y1, P2Y2, P2Y4, P2Y5, P2Y6, P2Y10, P2Y11) receptors. As shown by FURA-2 measurement, triggering of P2 receptors resulted in an increase in free intracellular Ca^{2+}. In combination with Tumor necrosis factor-α, ATP increased the expression of the DC surface markers CD80, CD83 and CD86 indicating a maturation promoting effect. DC expressed the ecto-apyrase CD39 and the ecto-5′-nucleotidase CD73 as demonstrated by RT-PCR. Extracellular ATP was rapidly hydrolyzed by these ecto-enzymes as shown by separation of ^{3}H-labeled ATP metabolites using a thin layer technique. These data suggest that ATP acts as a costimulatory factor on DC maturation.

Key words: Dendritic cell; ATP; P2 receptor; Ecto-nucleotidase

1. Introduction

Dendritic cells (DC) are the most potent antigen presenting cells of the immune system. In their immature stage they reside in peripheral tissues. Upon encountering antigen, like pathogens, they migrate to lymphoid organs where they trigger a specific T cell response [1]. Recently, DC have also come to represent a very interesting cell type for gene therapy and vaccination strategies against tumors and viral infections [2]. In vitro, immature DC can be generated from peripheral blood monocytes by culturing them in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin (IL)-4 [3,4]. These cells show high endocytic, but low T cell stimulatory capacity. Upon addition of monocyte conditioned medium [5] or a cocktail (IL-1β, IL-6, tumor necrosis factor α (TNF-α) and prostaglandin E2 (PGE2)) that mimics it, these DC develop into fully mature immunostimulatory cells [6].

Extracellular ATP affects the function of many tissues and cell types and has also been shown to exert immunomodulatory functions. It regulates, for example, the function of B and T cells [7,8] and induces cell death in macrophages [9]. ATP can be released by regulated exocytosis of vesicles and granules or by traumatic lysis of intact cells [10]. ATP mediates its effects by binding to P2 nucleotide receptors [11–13]. These belong to two main classes: G protein coupled metabotropic P2Y receptors and ionotropic ATP gated channels termed P2X receptors [11]. Both classes comprise a number of subtypes [14]. Signaling through P2Y receptors results in mobilization of inositol 1,4,5-triphosphate sensitive Ca^{2+} stores, activation of plasma membrane influx pathways and stimulation of diglyceride dependent protein kinase C enzymes [15]. Signaling through P2 receptors is terminated by hydrolysis of ATP by ecto-ATPases degrading ATP to ADP, ecto-apyrases catabolizing ATP and ADP to AMP and ecto-5′-nucleotidases catabolizing AMP into adenosine [16,17]. CD73, originally identified on B cells, has been found to be the major ecto-5′-nucleotidase [18], CD39, another B cell surface marker, is an ecto-apyrase [19].

Epidermal Langerhans cells have previously been shown to express membrane ATPase which appears to protect them against the cytotoxic effects of extracellular ATP [20–22]. Very little, however, is known about the expression and function of P2 receptors and ATPases on DC. Only recently it has been shown that DC express functional P2Y receptors [23]. In the present study we show the expression and functional characterization of several P2X (P2X1, P2X4, P2X5, P2X7) and P2Y (P2Y1, P2Y2, P2Y4, P2Y5, P2Y6, P2Y10, P2Y11) receptors as well as ATPases and 5′-ecto-nucleotidases on human DC during different maturation stages.

2. Materials and methods

2.1. Culture medium

RPMI 1640 (Bio Whittaker, Verviers, Belgium) was supplemented with 2 mM L-glutamine (Life Technologies, Karlsruhe, Germany), 100 IU/ml penicillin (Sigma, Deisenhofen, Germany), 100 μg/ml streptomycin (Sigma), 10 mM HEPES pH 7.0 (Sigma) and 10% heat inactivated fetal calf serum (PAA, Cölbe, Germany) (also termed complete medium).

2.2. Cytokines and ATP

Recombinant human cytokines were used in the following final concentrations: GM-CSF (Leukomax, Novartis, Basel, Switzerland) 800 U/ml, IL-4 (Novartis, Vienna, Austria) 1000 U/ml, IL-1β (Sigma) 10 ng/ml, IL-6 (Sandoz, Basel) 1000 U/ml, PGE2 (Sigma) 1 μg/ml, TNF-α (Bender, Vienna, Austria) 25 ng/ml. ATP (Sigma) was used at a concentration of 100 μM.

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Abbreviations: DC, dendritic cell; EGTA, ethylene glycol-*O*,*O*′-bis(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid); FITC, fluorescein isothiocyanate; GM-CSF, granulocyte-macrophage colony stimulating factor; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-4, interleukin 4; PGE2, prostaglandin E2; TNF-α, tumor necrosis factor α

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2.3. Antibodies

For immunostaining, mouse IgG CD14 (Dianova, Hamburg, Germany), CD80 (Immunotech, Marseille, France), CD83 (Immunotech), CD86 (Dianova) and mouse IgG subclass specific isotypes were used. Secondary antibody was biotinylated anti-mouse IgG (Amersham Pharmacia Biotech, Freiburg, Germany), staining was performed with streptavidin conjugated FITC (Amersham Pharmacia Biotech). Alternatively FITC or PE conjugated CD14 and CD83 and the isotype specific FITC and PE conjugated mouse IgG were used.

2.4. Oligonucleotides

For PCR amplification, the following gene specific primers were used: P2X2 sense 5′-CTG TGA AGA CGT GTG AGA TCT TTG G-3′, P2X2 antisense 5′-TTG AAG AGG TGA CGG TAG TTG GTC C-3′; P2X4 sense 5′-GAG ATT CCA GAT GCG ACC-3′; P2X4 antisense 5′-GAC TTG AGG TAA GTA GTG G-3′; P2X5 sense 5′-TCG ACT ACA AGA CCG AGA AG-3′; P2X5 antisense 5′-CTT GAC GTC CAT CAC ATT G-3′ [24]; P2X7 sense 5′-AAC ATC ACT TGT ACC TTC C-3′; P2X7 antisense 5′-TGT GAA GTC CAT CGC AGG-3′; P2Y1 sense 5′-CTA CAT CTT GGT ATT CAT CAT CGG-3′, P2Y1 antisense 5′-GAG ACT TGC TAG ACC TCT TGT CAC C-3′ [25]; P2Y2 sense 5′-CTC TAC TTT GTC ACC ACC AGC GCG-3′, P2Y2 antisense 5′-TTC TGC TCC TAC AGC CGA ATG TCC-3′ [25]; P2Y4 sense 5′-CCA CCT GGC ATT GTC AGA CAC C-3′; P2Y4 antisense 5′-GAG TGA CCA GGC AGG GCA CGC-3′ [26]; P2Y5 sense 5′-TGG TTA ACT GTG ATC GGA GG-3′; P2Y5 antisense 5′-AGT CAC TTC TCC TGA CAG ACC-3′; P2Y6 sense 5′-CGC TTC CTC TTC TAT GCC AAC C-3′; P2Y6 antisense 5′-CCA TCC TGG CGG CAC AGG CGG C-3′ [26]; P2Y10 sense 5′-CAT CAG TCT TCA AAG GTG C-3′; P2Y10 antisense 5′-GGA CAA CTG CTA ATG ATG G-3′; P2Y11 sense 5′-CAG CGT CAT CTT CAT CAC C-3′; P2Y11 antisense 5′-GCT ATA CGC TCT GTA GGC-3′; CD73 sense 5′-CAC CAA GGT TCA GCA GAT CCG C-3′, CD73 antisense 5′-GTT CAT CAA TGG GCG ACC GG [25]; CD39 sense 5′-GAC CCA GAA CAA AGC ATT GCC-3′, CD39 antisense 5′-TGT AGT CCT TGC CAG AGA GGC G-3′ [25]; GAPDH sense 5′-CAC CAC CAT GGA GAA GGC TGG-3′, GAPDH antisense 5′-GAA GTC AGA GGA GAC CAC CAG-3′. Oligonucleotides were obtained from MWG Biotech (Ebersberg, Germany) or Amersham Pharmacia Biotech.

2.5. Cell preparation

Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats using Lymphoprep (Nycomed Pharma, Oslo, Norway). Cells were plated onto IgG (Sigma) coated bacteriological 10 cm petri dishes (Falcon, Germany) at a concentration of 50×10^6/ml (Kruse et al., manuscript in preparation). After 45 min, non-adherent cells were rinsed off the dishes. Eight hours later medium was replaced by fresh medium supplemented with 800 U/ml GM-CSF and 1000 U/ml IL-4. On day 3 cells were fed with fresh medium (+5 ml) containing 400 U/ml GM-CSF and 500 U/ml IL-4. On day 4 or 5, non-adherent cells were rinsed off the dishes, washed once and transferred to tissue culture dishes at a density of 250 000 cells/ml. The next day, cells were stimulated with different combinations of cytokines and ATP. Macrophages were generated by plating PBMC onto IgG coated bacteriological dishes and culturing the adherent fraction in complete medium supplemented with 800 U GM-CSF/ml. After 7 days adherent cells were harvested.

2.6. Flow cytometry analysis

Staining and washing was performed in PBS (136 mM NaCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl pH 7.4) plus 1% FCS. Cells were incubated for 30 min on ice with each antibody in the appropriate dilution. After washing once, cells were incubated for 20 min with the secondary antibody and for 15 min with streptavidin-FITC. After washing once with PBS cells were analyzed by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA, USA).

2.7. RT-PCR

Total RNA was isolated using TriFast reagent (Peqlab, Erlangen, Germany) according to the manufacturer's instructions and digested with RNase free DNase I (Life Technologies). DNase treated RNA (2 μg) was reverse transcribed into cDNA using SuperScript II (Life Technologies). cDNA reactions were performed for 1 h at 42°C and stopped by boiling for 5 min. 2 μl of cDNA was used as a template for PCR with primers specific for P2X1, P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4, P2Y5, P2Y6, P2Y10, P2Y11, CD73 and CD39. As a control for cDNA synthesis, GAPDH-PCR was performed. PCR reactions included 0.5 μM of each primer, 200 μM of dNTP, 1 U Taq polymerase (Amersham Pharmacia Biotech) in the supplied reaction buffer. The PCR cycling conditions were as follows: for P2X1 receptor 45 s at 94°C, 45 s at 64°C, 1 min 30 s at 72°C (amplification product 463 bp); for P2X4, P2X7 and P2Y5 receptors 45 s at 94°C, 45 s at 54°C, 1 min at 72°C (amplification products: P2X4 296 bp, P2X7 675 bp, P2Y5 520 bp); for P2X5 receptor 45 s at 94°C, 45 s at 57°C, 45 s at 72°C (amplification product 596 bp); for P2Y1 receptor 45 s at 94°C, 45 s at 64°C, 1 min 30 s at 72°C (amplification product 772 bp); for P2Y2 receptor 45 s at 94°, 45 s at 67°C, 1 min 30 s at 72°C (amplification product 637 bp); for P2Y4 and P2Y6 receptor 45 s at 94°C, 45 s at 56.5°C, 1 min at 72°C (amplification products: P2Y4 425 bp, P2Y6 365 bp); for P2Y10 receptor 45 s at 94°C, 45 s at 52°C, 1 min at 72°C (amplification product 456 bp); for P2Y11 receptor 45 s at 94°C, 45 s at 57°C, 1 min at 72°C (amplification product 273 bp); for CD39 45 s at 94°C, 45 s at 62°C, 1 min 30 s at 72°C (amplification product 632 bp); for CD73 45 s at 94°C, 45 s at 64°C, 1 min 30 s at 72°C (amplification product 1006 bp); for GAPDH 45 s at 94°C, 45 s at 60°C, 1 min 30 s at 72°C (amplification product 553 bp). All PCRs were carried out for 35 cycles and included an initial 3 min denaturation step at 94°C and a final 10 min extension at 72°C.

2.8. Intracellular free [Ca^{2+}] measurements

Free intracellular calcium was measured using the fluorescence indicator FURA-2AM as described [27]. Briefly, 5×10^6 DC were harvested on day 7, centrifuged and resuspended in 1 ml RPMI supplemented with 10% FCS. While shaking gently, 4 μl FURA-2AM (stock: 1 mM in DMSO) at a final concentration of 4 μM was added. Cells were incubated 15 min at 37°C, then diluted 1/5, centrifuged ($300\times g$), washed once and resuspended in 6 ml medium for Ca^{2+} measurement (RPMI, 140 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 20 mM HEPES, 1 mM NaH_2PO_4 and 5.5 mM glucose, pH 7.4). Fluorescence in a 2 ml suspension was determined in a LS-3B fluorometer (Perkin Elmer, Überlingen, Germany) equipped with a chart recorder. Excitation wavelength was 340 nm, emission wavelength was 510 nm. All measurements were carried out at room temperature. Maximal fluorescence was determined by addition of 0.1% Triton X-100, minimal fluorescence by addition of 20 μl EGTA/Tris (4 mM EGTA and 40 mM Tris, pH 7.4).

2.9. Assay of nucleotide metabolism by cellular ecto-enzymes

For measurement of membrane bound nucleotide metabolizing enzyme activities, DC were harvested and washed twice in HBSS (Hanks' balanced salt solution). 2×10^5 cells in 50 μl HBSS were mixed with 50 μl HBSS containing 5 μM [^{3}H]ATP or 5 μM [^{3}H]AMP. 3 μl of cell suspension supernatant was spotted directly and at indicated timepoints onto PEI-cellulose thin layer sheets, a procedure that was found to interrupt all enzymatic activities in less than 30 s [28]. A mixture of marker nucleotides was added to each spot on the sheet. Separation was achieved with water and after drying with 0.85 M LiCl at room temperature. After drying, separated nucleotides were marked under UV light and excised. Radioactivity was determined in a scintillation mixture (toluene, 0.5% 2,5-diphenyloxazol, 0.03% dimethyl-[1,4-bis(4-methyl-5-phenoxyloxazol-2-yl)benzene]) in a β-counter.

3. Results

3.1. Expression of P2X and P2Y receptors in DC

To analyze the expression of P2X and P2Y receptors in DC generated from buffy coats, cells were either left untreated or stimulated with ATP alone or with a maturation cocktail (IL-1β, IL-6, TNF-α and PGE2) or with a combination of cocktail and ATP. To investigate receptor expression on immature DC, cells were harvested on day 4 without stimulation and controlled using FACS analysis. After stimulation with the maturation cocktail, more than 85% of cells expressed CD83 whereas among untreated and immature cells less than 20%

were positive for this marker. Total RNA was isolated and RT-PCR was performed with primers specific for P2X1, P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4, P2Y5, P2Y6, P2Y10 and P2Y11. Apart from DC, the following five cell types were also analyzed for their receptor expression: LB23 melanoma cells, Jurkat T cells, HL-60 promyelocytes, macrophages and DO11, a mouse T cell hybridoma cell line which served as a control for the specificity of the primers. Whereas myeloid cells have been reported to express either P2Y1 or P2Y2 receptors depending on their developmental stage [25], DC express all P2Y receptors analyzed as well as P2X receptors independent of stimulation (Table 1) and degree of maturity.

3.2. ATP enhances the maturation inducing effect of TNF-α

During maturation, DC upregulate the expression of the surface markers CD80, CD83 and CD86. To investigate whether ATP has an effect on this upregulation, DC were stimulated on day 5 or 6 either with ATP or with TNF-α alone or with a combination of TNF-α and ATP or left untreated. Twenty-four hours later FACS analysis was performed. Whereas ATP alone only modestly upregulated the expression of CD80, CD83 and CD86, the combination of ATP and TNF-α was clearly superior to TNF alone in inducing the expression of these maturation markers (Fig. 1).

3.3. Dendritic cells respond to extracellular ATP with intracellular Ca^{2+} increase

Most P2Y receptors couple to G proteins thus activating phospholipase C. This leads to the cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-triphosphate (IP_3) which releases Ca^{2+} from intracellular stores and diacylglycerol thus activating protein kinase C (PKC). Increase of intracellular Ca^{2+} was used as a readout for P2Y-receptor signaling in DC. Cells were loaded with the fluorescence indicator FURA-2 as described above. The addition of ATP or other nucleotides at indicated concentrations to immature DC led to an increase of fluorescence intensity reflecting release of Ca^{2+} from intracellular stores (Fig. 2, upper panel). DC responded to 10 μM ATP with a rapid short increase of fluorescence intensity whereas a higher concentration of ATP (100 μM) led to a concentration dependent prolongation of the fluorescence peak (Fig. 2). The first phase is mainly due to Ca^{2+} release from intracellular stores, the second reflects Ca^{2+} influx across the plasma membrane. As previously shown for macrophages [9], these data suggest the presence of two receptor subtypes on DC: P2Y receptors mediating IP3 formation and mobilization of intracellular Ca^{2+} resulting in a short peak of increased fluorescence intensity, and at higher concentrations P2X receptor signaling leading to a pore forming ion channel for extracellular Ca^{2+}. This hypothesis is supported by the finding that complexing extracellular Ca^{2+} by equimolar concentrations of EGTA abolished the second phase of the response (Fig. 2, upper panel).

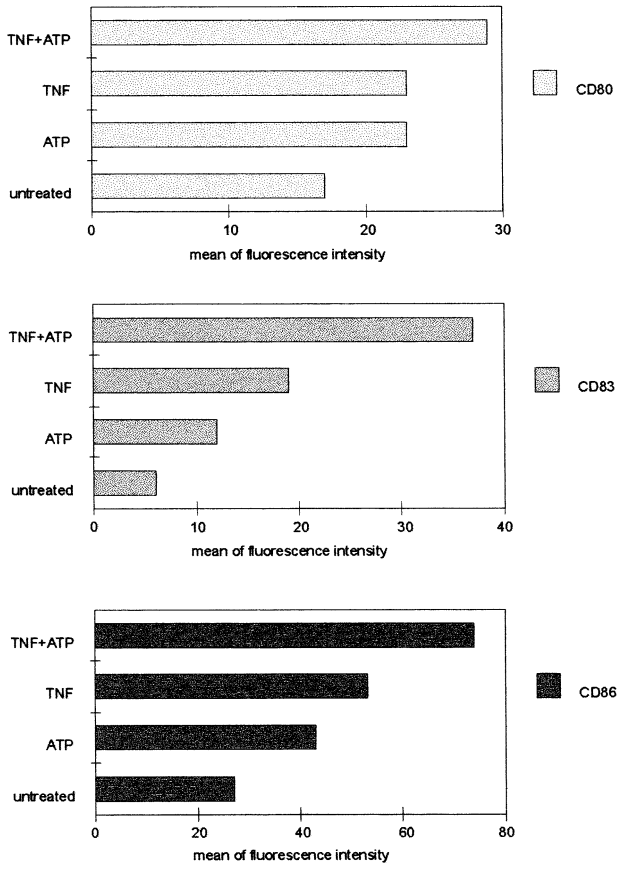

Fig. 1. Phenotypic analysis of differently stimulated DC. DC obtained from buffy coats were cultured as described. One day after transfer cells were stimulated as indicated or left untreated. On the following day cells were stained with antibodies against CD80, CD83 and CD86 and FACS analysis was performed. The mean fluorescence intensity of one representative experiment out of five is shown.

Table 1
Expression analysis of P2X and P2Y receptors

	P2X1	P2Y1	P2Y2	P2X4	P2X5	P2X7	P2Y4	P2Y5	P2Y6	P2Y10	P2Y11
LB23	+	−	+	+	+	−	+	−	+	−	+
Jurkat	+	+	−	+	+	+	+	+	−	+	+
Macrophages	+	+	+	+	+	+	+	+	+	+	+
DO11	−	−	−	−	−	−	−	+	−	−	−
HL-60	+	−	+	+	−	+	+	+	+	−	+
DC d4	+	+	+	+	+	+	+	+	+	+	+
DC d7	+	+	+	+	+	+	+	+	+	+	+
DC+ATP	+	+	+	+	+	+	+	+	+	+	+
DC+TIIP	+	+	+	+	+	+	+	+	+	+	+
DC+TIIP+ATP	+	+	+	+	+	+	+	+	+	+	+

RNA was extracted from the indicated cells and cell lines. DC were harvested either on day 4 (DC d4) or on day 7 (DC d7) and stimulated with ATP (+ATP), TNF-α+IL-1β+IL-6+PGE2 (+TIIP), or TNF-α+IL-1β+IL-6+PGE2+ATP (+TIIP+ATP). RT-PCR was performed with primers specific for the indicated P2X and P2Y receptors.

Next we investigated whether there was a difference in P2X and P2Y receptor signaling between immature and mature DC. As indicated in Fig. 2 (lower panel) the second phase of response to ATP triggering at a high concentration (100 μM) was significantly longer in mature DC when compared with immature DC. Complexing extracellular Ca^{2+} by EGTA again abrogated the second phase of response indicating that it was due to the influx of extracellular Ca^{2+} (Fig. 2, bottom panel). The rapid increase in intracellular Ca^{2+} was lower in immature DC (Fig. 2, upper panel). These results are indicative of a stage specific regulation of P2X and P2Y receptors during the differentiation of DC from blood precursor cells.

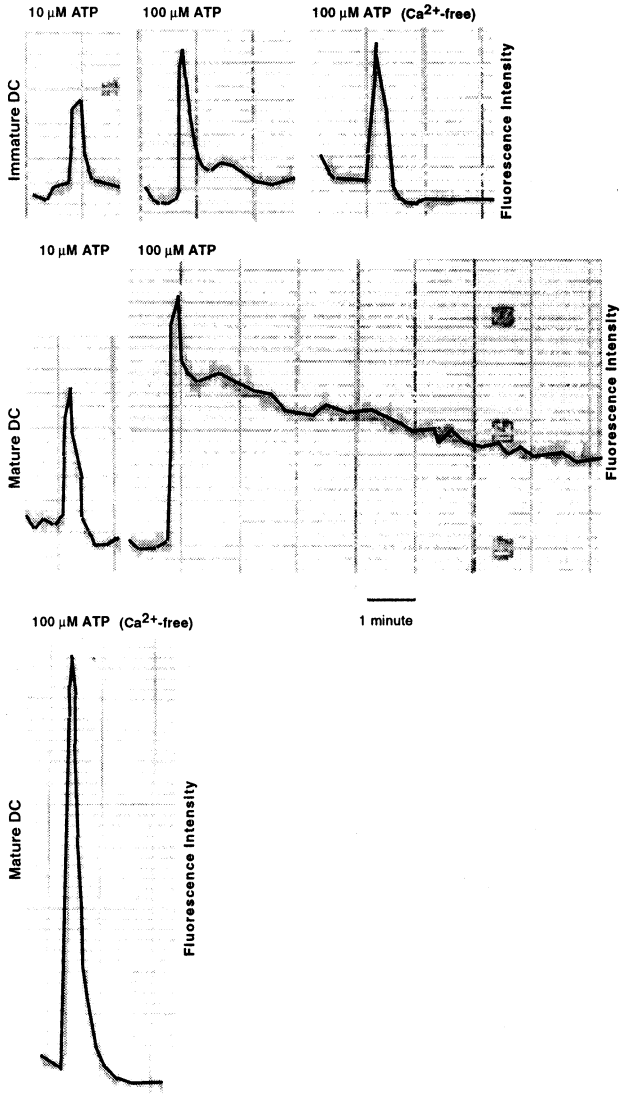

Fig. 2. Extracellular ATP activates P2Y and P2X receptors. Monocyte derived DC were either cultured with GM-CSF and IL-4 alone (immature, upper panel) or stimulated for 2 days with the maturation cocktail (IL-1β, IL-6, TNF-α and PGE2) (mature, lower panel). Cells were loaded with FURA-2 and ATP was added at the indicated concentrations. Changes in fluorescence intensity reflecting levels of free intracellular Ca^{2+} over the time are shown. Ca^{2+} free medium was obtained by addition of equimolar concentrations of EGTA. Graph is superimposed by a computer assisted smoothed line.

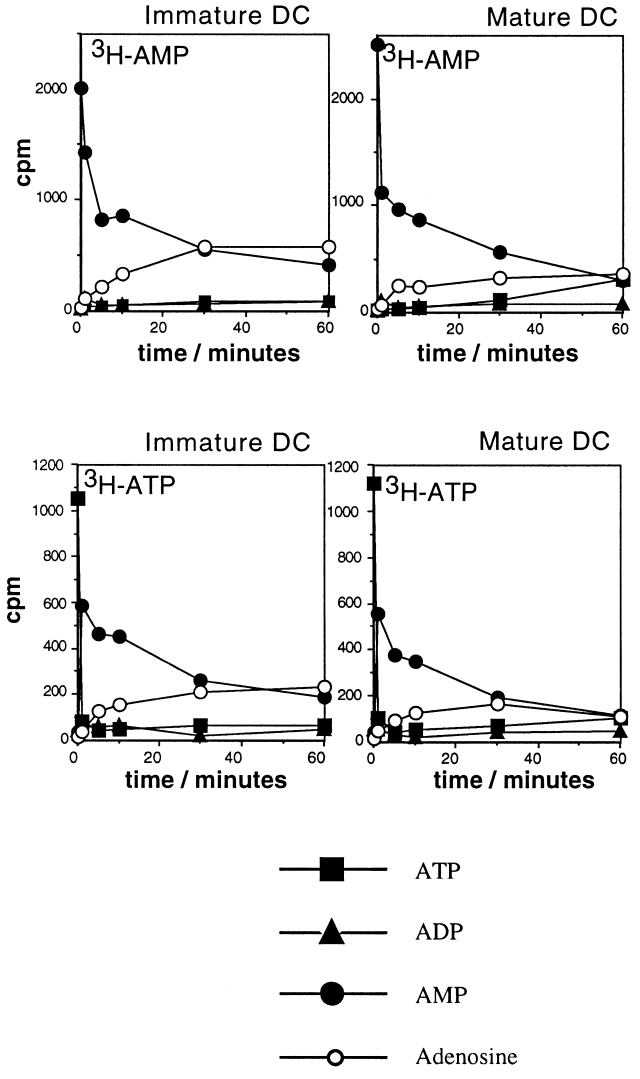

Fig. 3. Degradation of extracellular ATP and AMP by DC. DC were cultured with GM-CSF and IL-4 alone (immature DC, left panel) or stimulated with the maturation cocktail (IL-1β, IL-6, TNF-α, PGE2) for 48 hours (right panel). Cells were incubated with [^{3}H]AMP (upper panel) or [^{3}H]ATP (lower panel). At indicated timepoints, aliquots were taken and the metabolites [^{3}H]ADP, [^{3}H]AMP and [^{3}H]adenosine were measured. Amounts of metabolites are shown in cpm.

3.4. DC express mRNA for ATP and AMP metabolizing enzymes

Signaling through P2 receptors is terminated through the hydrolysis of extracellular ATP by ecto-ATPases and ecto-apyrases. Ecto-ATPases hydrolyze ATP to ADP, ecto-apyrases catabolize ADP and ATP to AMP, which is then catabolized to adenosine by the ecto-5′-nucleotidase. CD73 has been identified as the major ecto-5′-nucleotidase, whereas CD39 is an ecto-apyrase. RT-PCR analyses were performed to investigate whether DC express mRNA for both enzymes. As shown in Table 2, mRNA for CD39 and CD73 was detectable in DC on day 4 and day 7 independent of further cytokine stimulation. CD73 mRNA was also found in all other cell lines tested except for the mouse cell line, CD39 mRNA was not detectable in LB23 and in the mouse cell line.

Table 2
Expression analysis of CD39 and CD73

	CD39	CD73
LB23	−	+
Jurkat	+	+
Macrophages	+	+
DO11	−	−
HL-60	+	+
DC d4	+	+
DC d7	+	+
DC+ATP	+	+
DC+TIIP	+	+
DC+TIIP+ATP	+	+

RNA was extracted from the indicated cells and cell lines. DC were treated and harvested as in Table 1. RT-PCR was performed with primers specific for CD39 and CD73.

3.5. DC degrade extracellular ATP and AMP

For functional analyses, DC were incubated with [^{3}H]ATP or AMP. At indicated timepoints, aliquots of the cell suspension were spotted onto thin layer chromatography sheets and the degradation products [^{3}H]ADP, [^{3}H]AMP and [^{3}H] adenosine were separated and measured as described above. As shown in Fig. 3, DC show a very high activity of ATPase independent of their maturation stage (see upper and lower panels). After 1 min, [^{3}H]ATP was almost undetectable. Interestingly, the hydrolysis of ATP did not lead to an increase in ADP but in AMP and adenosine indicating the presence of a pyrophosphate hydrolase. When [^{3}H]AMP was added as a starter nucleotide to investigate the activity of an ecto-5′-nucleotidase, it was rapidly catabolized resulting in an increase in adenosine. There was no difference in enzyme activity between mature and immature DC (see upper and lower panel).

4. Discussion

In the present study we demonstrate that human monocyte derived DC express functional P2 receptors. By RT-PCR we show for the first time that DC express mRNA for P2Y and P2X receptors. This finding is underlined by functional studies where, in contrast to Liu et al. [23], we could detect not only metabotropic P2Y receptors but also ionotropic P2X receptors. In contrast to other myeloid cells where there is a clear stage specific expression of P2Y receptors [25], on human DC P2Y and P2X receptors were simultaneously expressed independent of their maturation stage. In functional analyses, however, we found increased Ca^{2+} release after the maturation stimulus as well as prolonged Ca^{2+} influx through the plasma membrane. This might indicate quantitative changes in receptor expression during the maturation of DC. In addition we found an additive effect of ATP and TNF-α on the upregulation of the cell surface markers CD80, CD83 and CD86 during the maturation of DC. As ATP alone shows only moderate effects it is most likely that ATP acts as a cofactor for DC maturation.

Extracellular ATP is very rapidly hydrolyzed by DC thus protecting the cells from ATP induced apoptosis [22] which is mediated by the P2X7 receptor [29] as well as from uncontrolled stimulation. RT-PCR clearly showed the expression of the ecto-5′-nucleotidase CD73 and the ecto-apyrase CD39. Again in contrast to other myeloid leukocytes where the expression is developmentally regulated [25], DC express both enzymes during all maturation stages. This is supported by functional analyses where no differences in activity were observed. The expression of these putative ecto-enzymes by DC at all stages of their differentiation might be important to protect these crucial cells at their immature antigen processing stage in the peripheral tissues as well as their mature, T cell sensitizing stage in the lymphoid organs.

In summary, these findings underline that ATP receptors and ATPases play an important role during differentiation and maturation of DC, which are influenced by the nucleotide concentration and the inflammatory cytokines.

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References

[1] Banchereau, J. and Steinman, R. (1998) Nature 392, 245–252.
[2] Schuler, G. and Steinman, R.M. (1997) J. Exp. Med. 186, 1183–1187.
[3] Romani, N., Gruner, S., Brang, D., Kämpgen, E., Lenz, A., Trockenbacher, B., Konwalinka, G., Fritsch, P.O., Steinman, R.M. and Schuler, G. (1994) J. Exp. Med. 180, 83–93.
[4] Sallusto, F. and Lanzavecchia, A. (1994) J. Exp. Med. 179, 1109–1118.
[5] Romani, N., Reider, D., Heuer, M., Ebner, S., Kampgen, E., Eibl, B., Niederwieser, D. and Schuler, G. (1996) J. Immunol. Methods 196, 137–151.
[6] Jonuleit, H., Kühn, U., Müller, G., Steinbrink, K., Paragnik, L., Schmitt, E., Knop, J. and Enk, A.H. (1997) Eur. J. Immunol. 27, 3135–3142.
[7] Padeh, S., Cohen, A. and Roifman, C.M. (1991) J. Immunol. 146, 1626–1632.
[8] Baricordi, O.R., Ferrari, D., Melchiorri, L., Chiozzi, P., Hanau, S., Chiari, E., Rubini, M. and Di Virgilio, F. (1996) Blood 87, 682–690.
[9] Falzoni, S., Munerati, M., Ferrari, D., Spisani, S., Moretti, S. and Di Virgilio, F. (1995) J. Clin. Invest. 95, 1207–1216.
[10] Gordon, J.L. (1986) Biochem. J. 233, 309–319.
[11] Dubyak, G.R. and El-Moatassim, C. (1993) Am. J. Physiol. 265, C577–C606.
[12] Fredholm, B.B. (1994) Pharmacol. Rev. 46, 143–156.
[13] Burnstock, G. (1997) Neuropharmacology 36, 1127–1139.
[14] Burnstock, G. and King, B.F. (1996) Drug Dev. Res. 38, 67–71.
[15] Harden, T.K., Boyer, J.L. and Nicholas, R.A. (1995) Annu. Rev. Pharmacol. Toxicol. 35, 541–579.
[16] Komszynski, M. and Wojtczak, A. (1996) Biochim. Biophys. Acta 1310, 233–241.
[17] Plesner, L. (1995) Int. Res. Cytol. 158, 141–214.
[18] Zimmermann, H. (1992) Biochem. J. 285, 345–365.
[19] Wang, T. and Guidotti, G. (1996) J. Biol. Chem. 271, 9898–9901.
[20] Wolff, K. (1964) Arch. Klin. Exp. Dermatol. 218, 254–273.
[21] Wolff, K. and Winkelmann, R.K. (1967) J. Invest. Dermatol. 48, 50–54.
[22] Girolomoni, G., Santantonio, M.L., Pastore, S., Bergstresser, P.R., Giannetti, A. and Cruz Jr., P.D. (1993) J. Invest. Dermatol. 100, 282–287.
[23] Liu, Q.H., Bohlen, H., Titzer, S., Christensen, O., Diehl, V., Hescheler, J. and Fleischmann, B.K. (1999) FEBS Lett. 445, 402–408.
[24] Le, K.T., Paquet, M., Nouel, D., Babinski, K. and Seguela, P. (1997) FEBS Lett. 418, 195–199.
[25] Clifford, E.E., Martin, K.A., Dalal, P., Thomas, R. and Dubyak, G.R. (1997) Am. J. Physiol. 273, C973–C987.
[26] Jin, J., Dasari, V.R., Sistare, F.D. and Kunapuli, S.P. (1998) Br. J. Pharmacol. 123, 789–794.
[27] Treves, S., Di Virgilio, F., Cerundolo, V., Zanovello, P., Collavo, D. and Pozzan, T. (1987) J. Exp. Med. 166, 33–42.
[28] Ogilvie, A., Lüthje, J., Pohl, U. and Busse, R. (1989) Biochem. J. 259, 97–103.
[29] Di Virgilio, F. (1995) Immunol. Today 16, 524–528.