Ecto-nucleoside Triphosphate Diphosphohydrolase 1 (E-NTPDase1/CD39) Regulates Neutrophil Chemotaxis by Hydrolizing Released ATP to Adenosine*

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Polymorphonuclear neutrophils release ATP in response to stimulation by chemoattractants, such as the peptide N-formyl-methionyl-leucyl-phenylalanine. Released ATP and the hydrolytic product adenosine regulate chemotaxis of neutrophils by sequentially activating purinergic nucleotide and adenosine receptors, respectively. Here we show that that ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1, CD39) is a critical enzyme for hydrolysis of released ATP by neutrophils and for cell migration in response to multiple agonists (N-formyl-methionyl-leucyl-phenylalanine, interleukin-8, and C5a). Upon stimulation of human neutrophils or differentiated HL-60 cells in a chemotactic gradient, E-NTPDase1 tightly associates with the leading edge of polarized cells during chemotaxis. Inhibition of E-NTPDase1 reduces the migration speed of neutrophils but not their ability to detect the orientation of the gradient field. Studies of neutrophils from E-NTPDase1 knock-out mice reveal similar impairments of chemotaxis in vitro and in vivo. Thus, E-NTPDase1 plays an important role in regulating neutrophil chemotaxis by facilitating the hydrolysis of extracellular ATP.

Release of ATP from cells and subsequent activation of plasma membrane P2 (purinergic/nucleotide) receptors play important physiological roles in many cell types (1–5). In addition to functioning as an autocrine/paracrine molecule that regulates cell physiology, ATP can be hydrolyzed by ecto-ATPases to generate adenosine (6, 7), which regulates cell function by the activation of P1 receptors (1). P1 receptors are G-protein-coupled receptors, whereas P2 receptors are of two types: P2Y receptors, which are G-protein-coupled receptors, and P2X receptors, which have ion channel activity (1). Release of ATP by human polymorphonuclear neutrophils (PMN)2 and activation of P2Y receptors by ATP and of P1 receptors by adenosine play a critical role in chemotaxis of PMN (8). ATP is released in a polarized manner at the leading edge of cells and amplifies chemotactic gradient signals by autocrine/paracrine feedback through P2Y receptors. Adenosine formed from released ATP stimulates cell migration toward the chemoattractant by activation of A3 adenosine receptors that accumulate at the leading edge of polarized PMN (8). The precise mechanisms by which PMN release and hydrolize ATP, thereby regulating the responsiveness of P2Y receptors and generating adenosine, have not been identified.

Several families of ecto-ATPases hydrolyze extracellular nucleotides in eukaryotic cells. The largest family, the ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases), consists of eight members (E-NTPDase1–8), which hydrolyze nucleotide triphosphates and diphosphates to monophosphates (10). The ecto-nucleotide pyrophosphatase/phosphodiesterases (E-NPPs) are the second largest family; these enzymes also hydrolyze nucleotide triphosphates to monophosphates and, in addition, they can convert cAMP to adenosine (11). Alkaline phosphatases (ALPs) are another family of ecto-ATPases, of which four isoforms have been identified (6), and finally, ecto-5′-nucleotidase (CD73), comprising one member only, catalyzes the conversion of AMP to adenosine (12). In the current study, we show that E-NTPDase1, which is also known as CD39, is expressed and catalyzes the hydrolysis of extracellular ATP by PMN and that this hydrolysis plays a critical role in chemotaxis.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were obtained from Sigma unless otherwise noted. Monoclonal anti-tissue nonspecific ALP antibody was obtained from AbCam (Cambridge, MA). Alexa Fluor 488 goat anti-mouse IgG secondary antibodies were obtained from Invitrogen.

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2 The abbreviations used are: PMN, polymorphonuclear neutrophils; fMLP, N-formyl-methionyl-leucyl-phenylalanine; E-NTPDase1, ecto-nucleoside triphosphate diphosphohydrolase 1; E-NPP, ecto-nucleotide pyrophosphatase/phosphodiesterase; IL-8, interleukin-8; ALP, alkaline phosphatase; HPLC, high pressure liquid chromatography; RT-PCR, reverse transcription-PCR; HBSS, Hanks’ balanced salt solution; WT, wild type; W-peptide, WKYMVM.
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**PMN Isolation**—The Beth Israel Deaconess Medical Center (BIDMC) and University of California San Diego (UCSD) Human Research Programs approved all experiments undertaken in this study. PMN were isolated from the peripheral blood of healthy volunteers as described previously (13). Briefly, blood was treated with 5% Dextran-500, dissolved in normal saline, and allowed to sediment at room temperature for 30 min. The cells in the supernatant were then separated by Percoll gradient centrifugation and washed as described previously (13).

**HPLC Analysis of Extracellular Nucleotides**—Cell culture supernatants were collected and analyzed using HPLC as described previously (8). Nucleotides were identified, and their concentrations were estimated using their retention times as compared with known standards.

**Malachite Green Assay**—Solutions of malachite green (0.812 g/liter deionized H2O), ammonium molybdate (0.3 M in 6 N HCl), and polyvinyl alcohol (23.2 g/liter H2O) were prepared as described previously (14). To produce the assay solution, 4 ml of malachite green solution was mixed with 2 ml each of the ammonium molybdate and polyvinyl alcohol solutions. The supernatants of cell suspensions were collected and mixed at a ratio of 1:1 with the malachite green assay solution. After incubation at room temperature for 5 min, samples were analyzed in a plate reader at 650 nm and compared with a KH2PO4 standard curve to obtain the concentration of inorganic phosphate.

**Immunofluorescence Staining**—PMN (2.5 × 10⁶/ml) were plated on glass coverslips and stimulated with 100 nM N-formyl-methionyl-leucyl-phenylalanine (fMLP) for 10 min. The cells were permeabilized for 5 min with 0.1% Triton X-100, fixed with 3.7% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature, and stained with antibodies using 500 ng/ml anti-tissue nonspecific ALP, 500 ng/ml anti-E-NTPDase1, and 4 μg/ml Alexa Fluor 488 goat anti-mouse IgG secondary antibodies, as described previously (9).

**Real-time RT-PCR**—Total RNA was extracted from PMN using a TRIzol extraction method (Invitrogen). First strand cDNA was synthesized using Superscript II RNase H-reverse transcriptase (Invitrogen). Real-time RT-PCR was performed using primer sets and the QuantiTect SYBR Green PCR kit (Invitrogen). Real-time RT-PCR was performed as described previously (9).

**Hydrolysis of Extracellular Nucleotides by PMN**—Like many other cell types, human PMN release ATP in response to a number of stimuli (15–17). ATP release in response to fMLP along with its the ATP hydrolytic product adenosine regulates polarization and migration of PMN (8). To evaluate the hydrolysis of extracellular nucleotides by PMN, we added ATP, ADP, or AMP (100 μM) to PMN (5×10⁶ in 0.25 ml of HBSS) and used HPLC to assess the concentrations of each nucleotide and their hydrolytic products after different incubation periods at 37°C. We found that PMN hydrolyzed 100 μM ATP by >80% within 10 min; AMP concentrations increased correspondingly, but concentrations of ADP remained low and changed little over time (Fig. 1A). These results imply that PMN hydrolyze extracellular ATP directly to AMP, generating negligible amounts of ADP in the process. Such catalytic properties are characteristic of E-NTPDase1 (6). Consistent with a role for this enzyme in ATP hydrolysis, we found that PMN efficiently hydrolyze extracellular ADP, reducing 100 μM ADP by ~60% within 10 min of incubation at 37°C (Fig. 1B). By contrast, PMN hydrolyzed <10% of 100 μM AMP under similar incubation conditions (Fig. 1C). Hydrolysis of AMP is thus a rate-limiting step in the generation of extracellular adenosine by PMN.

**Hydrolysis of Extracellular Nucleotides**—To determine which nucleotide-hydrolyzing enzyme(s) might mediate the hydrolysis of ATP, we used real-time RT-PCR analysis to evaluate the expression of ecto-nucleotidases in PMN and HL-60 cells, which were differentiated to neutrophil-like phenotype (Fig. 2). Based on the enzymatic activities determined above (Fig. 1), we focused our attention on members of the E-NTPDase enzyme family, which catalyze hydrolysis of ATP and ADP but not AMP, and on enzymes that generate adenosine (6). We detected the expression of two E-NTPDases that hydrolyze ATP to AMP (E-NTPDase1 and E-NTPDase2).
E-NTPDase2) and of tissue-nonspecific ALP, which, among other activities, hydrolyzes AMP to adenosine (6). We also found expression of two members of the E-NPP family (E-NPP1 and E-NPP2), although only one of these enzymes, E-NPP2, was expressed at significant levels. The relative expression levels of ecto-nucleotidases in differentiated HL-60 cells were similar to those in PMN with dominant mRNA expression of both E-NTPDase1 and E-NTPDase2 (Fig. 2B). HL-60 cells did not express tissue-nonspecific ALP but showed a higher relative expression of E-NPP2. E-NTPDase4 was highly expressed in both cell types (data not shown); however, this enzyme hydrolyzes UTP and UDP but not ATP (10).

Kinetics of Extracellular Nucleotide Breakdown by PMN—Four of the eight known E-NTPDases (E-NTPDase1, E-NTPDase2, E-NTPDase3, and E-NTPDase8) hydrolyze ATP (6). Each is also capable of hydrolyzing ADP, but the relative efficiency of breakdown of ATP versus ADP varies among the isoforms. Thus, the E-NTPDases can be distinguished by their enzymatic activity in hydrolyzing ATP versus ADP. As shown above, we found that two E-NTPDases, E-NTPDase1 and E-NTPDase2, are expressed by human PMN. The relative efficiency of ATP versus ADP breakdown by E-NTPDase1 is 1.0 versus 0.5–0.9 (18, 19, 20), whereas E-NTPDase2 has a stronger preference for ATP with a relative efficiency ratio of 1:0.03 (21–23). To determine whether E-NTPDase1 or E-NTPDase2 is more important for the hydrolysis of extracellular ATP by PMN, we examined the initial rates of hydrolysis of ATP and ADP (Fig. 3) and used Lineweaver-Burk plots to derive $K_m$ and $V_{max}$ values. PMN hydrolyzed ATP more effectively ($K_m = 20.9$ μM, $V_{max} = 9.48$ μmol of P$_i$/min/10$^6$ cells) than ADP ($K_m = 12.8$ μM, $V_{max} = 1.78$ μmol of P$_i$/min/10$^6$ cells). The enzymatic efficiencies for breakdown of ATP and ADP, derived by dividing $V_{max}$ by $K_m$, yielded a ratio of 1:0.31. This result implies that E-NTPDase1 likely plays a dominant role in the hydrolysis of extracellular ATP by PMN. Differentiated HL-60 cells also hydrolyzed ATP more effectively ($K_m = 2.46$ μM, $V_{max} = 0.96$ μmol of P$_i$/min/10$^6$ cells) than ADP ($K_m = 1.49$ μM, $V_{max} = 0.23$ μmol of P$_i$/min/10$^6$ cells). HL-60 cells, whereas overall not as efficient at hydrolyzing extracellular nucleotides as PMN, showed similar relative enzyme efficiencies for the breakdown of ATP and ADP (1:0.38).

PMN also hydrolyze AMP to adenosine, although at a much slower rate (Fig. 3A, inset), which did not make it possible to calculate $K_m$ and $V_{max}$. Under identical conditions, HL-60 cells did not exhibit sufficient AMP hydrolysis to generate a detectable signal.
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Inhibition of E-NTPDase1 Blocks ATP Hydrolysis—To further test whether E-NTPDase1 preferentially mediates the hydrolysis of extracellular ATP by PMN, we used two inhibitors of this enzyme: NaN₃, which inhibits E-NTPDase1 (24), and ARL67156, which inhibits E-NTPDase1 and 3 (25). Both NaN₃ and ARL67156 inhibited ATP hydrolysis in a concentration-dependent manner (Fig. 4). Thus, the real-time RT-PCR results, the kinetics of enzyme activity, and the results with E-NTPDase inhibitors are all consistent with the conclusion that E-NTPDase1 plays an important role in the hydrolysis of extracellular ATP by human PMN.

Inhibitors of E-NTPDase1 Inhibit PMN Chemotaxis—We assessed the contribution of E-NTPDase1-mediated hydrolysis of ATP to the regulation of chemotaxis of human PMN. Purified PMN migrated toward a point source of fMLP at an average speed of 4.7 ± 1.1 μm/min with an accuracy of gradient sensing, such that 94% of cells migrated toward the chemotactic source on paths that did not deviate >30° from a straight line (Fig. 5A). Inhibitors of ATP hydrolysis reduced the migration speed of PMN. ARL67156 reduced the average migration speed by 75% (1.2 ± 0.6 μm/min), but most cells (83%) maintained their migration path within 30° of a straight line toward the chemotactic source, indicating that gradient sensing is not substantially altered by inhibiting ATP hydrolysis. NaN₃ also

![Kinetics of nucleotide hydrolysis by PMN and HL-60 cells.](image)

![Inhibitors of E-NTPDase1 decrease nucleotide hydrolysis by PMN.](image)

![Inhibitors of E-NTPDase1 decrease PMN chemotaxis.](image)
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inhibits E-NTPDase1 activity; it reduced the average migration speed by 66% (1.6 ± 0.06 μm/min), whereas leaving gradient sensing largely intact (85% of cells maintained a correct migration path). The reduction in cell migration speed produced by the inhibitors leads us to conclude that a function of E-NTPDase1 is to regulate the velocity of PMN during chemotaxis.

Sodium metatungstate (POM1) is an inhibitor of E-NTPDases that inhibitors E-NTPDase1, E-NTPDase2, and E-NTPDase3 with $K_i$ values of 2.6, 3.3, and 28.8 μM, respectively (26, 27). We found that treatment of PMN with POM1 reduces chemotaxis at inhibitor concentrations that are consistent with E-NTPDase1 (Fig. 5B).

To evaluate the role of E-NTPDase1 in PMN responses to multiple classes of chemoattractants, we assessed the effects of ARL67156 on cell migration toward fMLP, IL-8, and zymosan-activated serum (C5a) using a Transwell assay system. Treatment with ARL67156 substantially reduced the PMN migration in response to all three agonists (Fig. 5C). Thus, E-NTPDase1 modulates PMN chemotaxis in response to multiple types of chemoattractants.

E-NTPDase1 Is Localized to the Leading Edge of Migrating PMN and HL-60 Cells—Given the functional role of E-NTPDase1 in regulating PMN chemotaxis, we used immunocytochemistry to determine the localization of this enzyme in human PMN. PMN were plated onto glass coverslips and stimulated with 10 nM fMLP for 2.5 min at 37 °C prior to fixation and staining with E-NTPDase1 antibodies. In 82% of polarized PMN, we observed accumulation of E-NTPDase1 at the leading edge (Fig. 6A); we found similar results in differentiated HL-60 cells (Fig. 6B). Thus, E-NTPDase1 is recruited to the leading edge during cell polarization. At the leading edge, this ecto-ATPase then hydrolyzes ATP that is most prominently released at this site during cell migration (8).

E-NTPDase1 Knock-out Mice Show Impaired PMN Migration—To study the role of E-NTPDase1 in the migration of PMN in an in vivo setting, we assessed the influx of PMN into the abdominal cavities of E-NTPDase1 knock-out mice and WT controls in response to formyl peptide receptor ligand W-peptide injected into the peritoneal cavity as described previously (8). As compared with an injection of saline vehicle control, injection of W-peptide increased the number of PMN that migrated in the peritoneal cavities of WT mice by 1.8-fold, whereas this response was significantly reduced in E-NTPDase1 knock-out mice (Fig. 7A).

Impaired migration of PMN from E-NTPDase1 knock-out mice was also observed in vitro using PMN isolated from the bone marrow of WT and E-NTPDase1 knock-out mice. The average migration speed of PMN from E-NTPDase1 knock-out mice (0.11 ± 0.03 μm/min) was 80% slower than that of PMN from WT controls (0.52 ± 0.15 μm/min), but PMN from the knock-out and WT mice showed no difference in their ability to polarize and migrate in the correct direction (Fig. 7B).

DISCUSSION

The results presented here extend previous findings regarding the role of polarized ATP release and the activation of P1/P2 receptors in the regulation of PMN chemotaxis—To study the role of E-NTPDase1 in the migration of PMN in an in vivo setting, we assessed the influx of PMN into the abdominal cavities of E-NTPDase1 knock-out mice and WT controls in response to formyl peptide receptor ligand W-peptide injected into the peritoneal cavity as described previously (8). As compared with an injection of saline vehicle control, injection of W-peptide increased the number of PMN that migrated in the peritoneal cavities of WT mice by 1.8-fold, whereas this response was significantly reduced in E-NTPDase1 knock-out mice (Fig. 7A).

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tant consequence of the latter effect would be to prevent aberrant polarization of PMN, which would occur by the activation of P2Y2 receptors that are not located at the leading edge, resulting in random migration rather than chemotaxis.

E-NTPDase1 catalyzes the first step in the production of adenosine, which activates A3 receptors, whose translocation to the leading edge of PMN promotes directional movement and defines migration speed (8). Adenosine also activates other receptors of PMN, such as A2a receptors, which are uniformly distributed and may help prevent leading edge formation at the trailing edge of migrating cells. Consistent with these ideas, we find that inhibition of E-NTPDase1 diminishes the migration speed of PMN in a gradient of fMLP. This inhibition is not unique to fMLP-promoted chemotaxis as inhibition of E-NTPDase1 also decreases the migration efficiency in response to gradients of IL-8 and C5a. In addition, we find that chemotaxis of PMN in E-NTPDase1 knock-out mice is impaired because of greatly reduced migration speed, although gradient sensing is maintained.

Although E-NTPDase1 plays an important role in ATP hydrolysis and PMN function, a second enzyme is required to complete the hydrolysis of ATP to adenosine. In human PMN, ALP, which is known to help regulate functions of neutrophils (30), is a likely candidate. Studies with inhibitors of ALP suggest that the enzyme contributes to the generation of extracellular adenosine and the control of PMN chemotaxis (data not shown). By contrast, differentiated HL-60 cells do not express ALP, although these cells are able to navigate chemotactic gradients. HL-60 cells express E-NPP2, also known as autotaxin, which can facilitate hydrolysis of AMP to adenosine and thus may perform a similar function as does ALP in PMN (10).

The current data highlight the critical role of E-NTPDase1 in modulation of PMN chemotaxis by regulation of ATP hydrolysis both in vitro and in vivo. Accordingly, E-NTPDase1 represents a potential therapeutic target for the treatment of inflammatory diseases that involve the aberrant or excessive accumulation of PMN in inflamed tissues.

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REFERENCES
1. Burnstock, G. (2006) Br. J. Pharmacol. 147, Suppl. 1, S172–S181
2. North, R. A., and Verkhratsky, A. (2006) Pfluegers Arch. Eur. J. Physiol. 452, 479–485
3. Schwiebert, E. M., and Zsombory, A. (2003) Biochim. Biophys. Acta 1615,
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7–32

4. Fredholm, B. B. (1997) *Gen. Pharmacol.* 28, 345–350
5. Robson, S. C., Sévigny, J., and Zimmermann, H. (2006) *Purinergic Signal.* 2, 409–430
6. Zimmermann, H. (2006) *Novartis Found. Symp.* 276, 113–128
7. Lazarowski, E. R., Boucher, R. C., and Harden, T. K. (2000) *J. Biol. Chem.* 275, 31061–31068
8. Chen, Y., Corriden, R., Boucher, R. C., and Harden, T. K. (2000) *J. Biol. Chem.* 275, 31061–31068
9. Chen, Y., Shukla, A., Namiki, S., Insel, P. A., and Junger, W. G. (2004) *J. Leukocryte Biol.* 76, 245–253
10. Zimmermann, H. (2000) *Handbook of Experimental Pharmacology* (Abbracchio, M. P., and Williams, M., eds) pp. 209–250, Springer, Berlin Heidelberg New York
11. Goding, J. W., Grobben, B., and Slegers, H. (2003) *Biochim Biophys Acta.* 1638, 1–19
12. Hunsucker, S. A., Mitchell, B. S., and Spychala, J. (2005) *Pharmacol Ther.* 107, 1–30
13. Junger, W. G., Hoyt, D., Davis, R. E., Herdon-Remelius, C., Namiki, S., Junger, H., Loomis, W., and Altman, A. (1998) *J. Clin. Investig.* 101, 2768–2777
14. Hohenwallner, W., and Wimmer, E. (1973) *Clin. Chim. Acta* 45, 169–175
15. Bodin, P., and Burnstock, G. (2001) *Neurochem. Res.* 26, 959–969
16. Yegutkin, G., Bodin, P., and Burnstock, G. (2000) *Br. J. Pharmacol.* 129, 921–926
17. Roman, R. M., Feranchak, A. P., Davison, A. K., Schwiebert, E. M., and Fitz, J. G. (1999) *Am. J. Physiol.* 277, G1222–G1230
18. Kaczmarek, E., Kozik, K., Sevigny, J., Siegel, J. B., Anrather, J., Beaudoin, A. R., Bach, F. H., and Robson, S. C. (1996) *J. Biol. Chem.* 271, 33116–33122
19. Wang, T. F., and Guidotti, G. (1998) *J. Biol. Chem.* 273, 11392–11399
20. Heine, P., Braun, N., Heilbronn, A., and Zimmermann, H. (1999) *Eur. J. Biochem.* 262, 102–107
21. Kegel, B., Braun, N., Heine, P., Maliszewski, C. R., and Zimmermann, H. (1997) *Neuropharmacology* 36, 1189–1200
22. Kegel, B., Braun, N., Heine, P., Maliszewski, C. R., and Zimmermann, H. (1997) *Neuropharmacology* 36, 1189–1200
23. Vlajkovic, S. M., Thorne, P. R., Housley, G. D., Muñoz, D. J., and Kendrick, I. S. (1998) *Hear. Res.* 117, 71–80
24. Crack, B. E., Pollard, C. E., Beukers, M. W., Roberts, S. M., Hunt, S. F., Ingall, A. H., McKechnie, K. C., Ilzerman, A. P., and Leff, P. (1995) *Br. J. Pharmacol.* 114, 475–481
25. Müller, C. E., Iqbal, I., Baqi, Y., Zimmermann, H., Röllich, A., and Stephan, H. (2006) *Bioorg Med Chem Lett.* 16, 5943–5947
26. Grenz, A., Zhang, H., Hermes, M., Eckle, T., Klingel, K., Huang, D. Y., Müller, C. E., Robson, S. C., Osswald, H., and Eltzschig, H. K. (2007) *FASEB J.* 21, 2863–2873
27. Enjoji, K., Sévigny, J., Lin, Y., Frenette, P. S., Christie, P. D., Esch, J. S. 2nd, Imai, M., Edelberg, J. M., Rayburn, H., Lech, M., Beeder, D. L., Csizmadia, E., Wagner, D. D., Robson, S. C., and Rosenberg, R. D. (1999) *Nat. Med.* 5, 1010–1017
28. Eltzschig, H. K., Eckle, T., Mager, A., Küper, N., Karcher, C., Weissmüller, T., Boengler, K., Schulz, R., Robson, S. C., and Colgan, S. P. (2006) *Circ. Res.* 99, 1100–1108
29. Okun, D. B., and Tanaka, K. R. (1978) *Am. J. Hematol.* 4, 293–299