Inhibition of platelet recruitment by endothelial cell CD39/ecto-ADPase: significance for occlusive vascular diseases

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During their 7-9 day lifespan in the circulation platelets are mainly responsible for maintaining the integrity of the vasculature. In thrombocytopenic states, there is an increase in vascular permeability and fragility, presumably due to absence of this platelet function. In sharp contrast, biochemical or physical injury in the coronary, carotid or peripheral arteries induces platelet activation and platelet recruitment, which can culminate in thrombotic vascular occlusion. Since there is one death every 33 s from vascular occlusion in the United States, this situation constitutes a major public health issue.

In the course of studying interactions between cells of the vascular wall and those in the circulation, we observed that platelets in close proximity to endothelial cells do not respond to agonists *in vitro*. Experiments initiated in the late 1980's cumulatively indicated that endothelial cell CD39 – an ecto-ADPase – was mainly responsible for this phenomenon. CD39 rapidly and preferentially metabolizes ADP released from activated platelets. ADP is the final common pathway for platelet recruitment and thrombus formation, and platelet aggregation and recruitment are abolished by CD39. Our current hypothesis is that CD39 will be a novel antithrombotic agent for treating high risk patients who have activated platelets in their circulation – the identifying characteristic of coronary artery occlusion and thrombotic stroke.

A recombinant, soluble form of human CD39 has been generated. This is solCD39, a glycosylated protein of 66 kDa whose enzymatic and biological properties are identical to the full-length form of the enzyme. In our *in vitro* experiments, solCD39 blocks ADP-induced human platelet aggregation, and inhibits collagen- and thrombin receptor agonist peptide-induced platelet reactivity. We studied solCD39 *in vitro* in a murine model of stroke, which was shown to be driven by excessive platelet recruitment. In studies with CD39 wild-type ($CD39^{+/+}$) mice solCD39 completely abolished ADP-induced platelet aggregation, and strongly inhibited collagen- and arachidonate-induced platelet reactivity *ex vivo*. When solCD39 was administered prior to transient intraluminal middle cerebral artery occlusion, it reduced ipsilateral fibrin deposition, decreased ^{111}In-platelet deposition, and increased post-ischemic blood flow 2-fold at 24 hours. These results were superior to those we obtained with aspirin pre-treatment.

CD39 null ($CD39^{-/-}$) mice, which we generated by deletion of exons 4-6 (apyrase conserved regions 2-4), have a normal phenotype, normal hematologic profiles and bleeding times, but exhibit a decrease in post-ischemic perfusion and an increase in cerebral infarct volume when compared to genotypic $CD39^{+/+}$ controls in our stroke model. "Reconstitution" of CD39 null mice with solCD39 reversed these pathologic changes. Thus, the $CD39^{-/-}$ mice were actually rescued from cerebral injury by solCD39, thereby fulfilling Koch's postulates. These experiments have led us to hypothesize that solCD39 has potential as a novel therapeutic agent for thrombotic stroke.

In this review, we summarize our recent research results with CD39 and solCD39, and discuss our viewpoints on its present and future possibilities as a novel treatment for thrombosis.

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Introduction

Cell-cell interactions in hemostasis and thrombosis. It is now recognized that thrombosis is a multicellular process[1-4]. When the morphology of evolving thrombi was initially studied by light and electron microscopy, erythrocytes, neutrophils, some monocytes, and platelets were all seen in close proximity. Platelets were also adherent to the exposed subendothelium, and this could be attributed to glycoprotein Ib and von Willebrand factor. Until recently, this multicellular occlusive mass, bound and consolidated by fibrin strands, was thought to represent a passive structure with cells randomly apposed, but not interacting biochemically (Fig. 1). Subsequent *in vitro*, *in vivo*, and *ex vivo* studies have verified that biochemical cell-cell interactions do

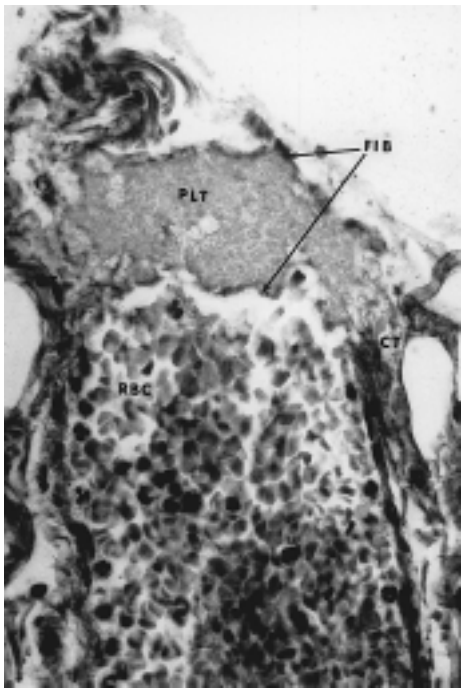

Figure 1. *Light microscopic view of thrombotic response to transsection of a mesenteric arteriole in the dog. A platelet plug (PLT) is seen surrounded by fibrin (FIB) and adherent to connective tissue (CT) exposed in the severed vessel. Erythrocytes (RBC) are seen in apposition to leukocytes and the platelet mass. We now know that extensive metabolic interchange occurs between all cells in the thrombus.*

occur in this setting. Platelets, neutrophils, erythrocytes, and endothelial cells are all active components in the microenvironment of the thrombus. The locution "transcellular metabolism" was originally suggested by Maclouf and Murphy[5] in 1988. Cell-cell interactions and transcellular metabolism in the evolving thrombus can result in metabolic generation of novel mediators[4-6]. Additionally, in the case of platelets and endothelial cells, such interactions can result in deletion by ecto-ADPase/CD39 of prothrombotic platelet-derived ADP[7,8]. Figure 2 schematically depicts transcellular metabolic interactions involving eicosanoid and nucleotidase pathways, both of which participate in generation of the evolving thrombus. Thus, arachidonic acid released from activated platelets (whether aspirin-treated or not) is utilized by other cells such as activated neutrophils, where it is processed to leukotriene B_4, one of the most proinflammatory substances yet described[6]. Activated platelets also release prostaglandin endoperoxides which are then transformed by endothelial cells (again, whether aspirin-treated or not) to prostacyclin, a strong platelet inhibitor and vasodilator[9,10]. Activated platelets also release ADP, the final common agonist in platelet activation and recruitment, which is metabolized to adenosine monophosphate (AMP) by CD39 on the endothelial cell surface. Subsequently, AMP is metabolized to adenosine by 5'-nucleotidase on the endothelial cell. This process resulting in inhibition of platelet recruitment forms the nidus for a new antithrombotic agent to treat occlusive vascular diseases.

Cell-cell interactions can be classified into three types (Table I). In type 1, different cells in proximity share common eicosanoid precursors. In type 2, a cell transforms an eicosanoid from a neighboring cell into a new metabolite that neither cell can synthesize alone. In type 3, an intermediate or eicosanoid generated by one cell serves as an agonist or inhibitor for biosynthesis of a different type of eicosanoid from a neighboring cell[11].

Thromboregulation. The cell-cell interactions described above can be categorized as participating in

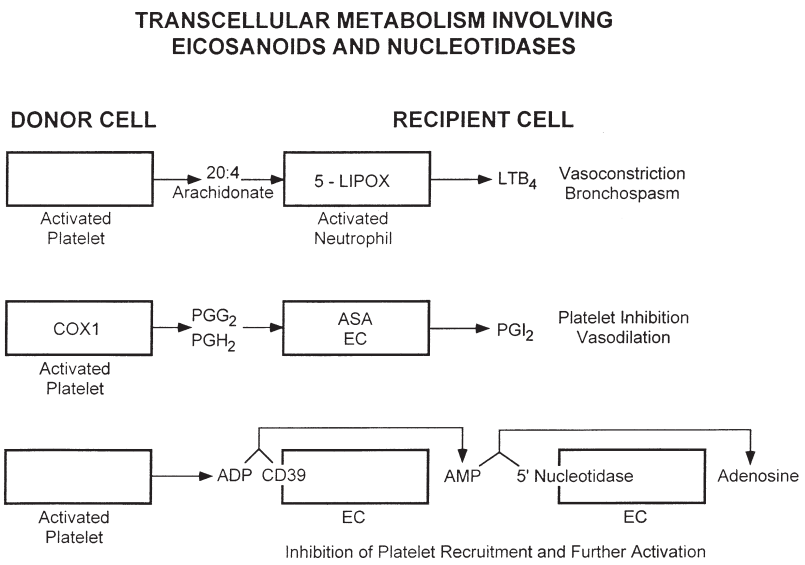

Figure 2. *Metabolites formed and released by activated platelets (donor) penetrate or interact with other cells, such as neutrophils and endothelial cells (EC), to form or be transformed into entirely new products with important (patho)physiological functions. ADP = adenosine diphosphate; AMP = adenosine monophosphate; ASA = acetylsalicylic acid; COX1 = cyclo-oxygenase-1; LT = leukotriene; PG = prostaglandin.*

Table I. Generation of new mediators via cell-cell interactions and transcellular metabolism in the eicosanoid pathway.

Type 1. Common eicosanoid precursors can be shared by different cells
- Type 1A. In addition to generating its own precursor, a cell can obtain the same compounds from another; more end product is thereby synthesized
- Type 1B. A cell cannot synthesize a precursor endogenously but can obtain it from a stimulated neighboring cell and use it for novel eicosanoid synthesis

Type 2. A cell can transform an eicosanoid from a neighboring cell into a new metabolite that neither cell alone can synthesize
- Type 2A. Both cells are activated by a common agonist
- Type 2B. An activated cell produces an eicosanoid that an unstimulated cell in proximity can use for generating a new metabolite

Type 3. An intermediate or eicosanoid generated by one cell can serve as an agonist or inhibitor for biosynthesis of a different type of eicosanoid from a neighboring cell
A leukotriene can serve as agonist for thromboxane production

"thromboregulation". We define thromboregulation as a process or group of processes by which circulating blood cells and cells of the vessel wall interact to regulate or inhibit thrombus formation. Virtually all thromboregulatory reactions are biochemical in nature and may result in formation of biologically active metabolites that could only have arisen through interactions between heterogeneous cell types in the vasculature[2,12-14]. Thromboregulation is accomplished by cell-associated or released compounds, which are either constitutive or elicited by stimuli (Table II).

We have classified vascular thromboregulators according to their chronological mode of action in relation to thrombin formation. Thus, the protein C-protein S natural anticoagulant system is operative after thrombin has formed at a site of vascular injury. In severely injured tissue, arterial thrombosis will occur because of the agonistic effect of the injury *per se* plus tissue factor release. Under these conditions, the injury site will escape thromboregulation. Vascular thromboregulators are classified in table III.

Inhibition of platelet activation and recruitment by endothelial cell ecto-ADPase/CD39. Recognition of the importance of vascular cell-cell interactions and transcellular metabolism in thrombosis and inflammation has become universally recognized in recent years[6,11,13,15-17]. This is quite pertinent with regard to platelets and endothelial cells. We believe that endothelial cells control platelet reactivity by at least four mechanisms (Table II): the cell-associated ecto-ADPase/CD39, and three fluid-phase reactants, the eicosanoids thromboxane A_2 and prostacyclin (PGI_2), as well as nitric oxide. The latter three reactants are generated by activated endothelium[11,18,19].

Table II. Thromboregulators.

Thromboregulator	Released	Aspirin-sensitive
Thromboxane	Yes	Yes
Prostacyclin	Yes	Yes
Nitric oxide	Yes	No
Ecto-ADPase/CD39 (E-NTPDase-1)	No	No

Table III. Classification of vascular thromboregulators.

Early thromboregulators (inhibit events preceding thrombin formation)
- Nitric oxide
- Eicosanoids (prostacyclin, PGD_2)
- Endothelial cell ecto-ADPase/CD39

Late thromboregulators (exert effects after thrombin formation)
- Antithrombin III
- Endothelial cell/heparin proteoglycans
- Tissue factor pathway inhibitor
- Thrombomodulin-protein C-protein S pathway
- Proteins of the fibrinolytic system

In previous studies, we could demonstrate inhibition of platelet aggregation by PGI_2 synthesized by aspirin-treated endothelial cells from released platelet endoperoxides[9]. We subsequently performed experiments in which nitric oxide production was neutralized by hemoglobin, and both platelets and endothelial cells were treated with aspirin inhibiting all PGI_2 production. These experiments indicated that the aspirin-treated, nitric oxide-deficient endothelial cells still inhibited platelet function via a mechanism involving metabolism of ADP[7].

We now propose that the molecule primarily responsible for platelet inhibition in the vasculature is the membrane-associated ecto-nucleotidase of the E type[20], now known as CD39. This enzyme metabolizes ATP and ADP to AMP. It is now classified as E-NTPDase-1 (Table IV)[8,22-35]. A simplified schematic of the domain structure of ecto-ADPase/CD39 is shown in figure 3.

Identification of the human endothelial cell ecto-ADPase as CD39. Before 1990, the prevailing hypothesis was that platelet inhibition by endothelial cells was due to endothelial cell eicosanoid and/or nitric oxide production. We tested this hypothesis by incubating aspirin-treated human umbilical vein endothelial cells (HUVECs) with radiolabeled ADP. Thus, PGI_2 formation was prevented, and, moreover, any nitric oxide generated was blocked by addition of purified oxyhemoglobin to the system. The fate of the added ADP and any metabolites generated was determined by radiothin layer chromatography (Fig. 4). These experiments demonstrated accumulation of AMP, which was further metabolized to adenosine by the 5'-nucleotidase on the endothelial cells, followed by intracellular deamination

Table IV. Nomenclature for E-type ecto-nucleotidases.

Name	Previously used names	Key references
NTPDase-1	CD39, ecto-ADPase, ecto-ATP diphosphohydrolase, ecto-apyrase (endothelium, activated B cells)	8,22-27
NTPDase-2	CD39L1, ecto-ATPase (brain)	28-30
NTPDase-3	CD39L3, HB6 (brain)	31,32
NTPDase-4	UDPase (component of brain Golgi lumen)	33
NTPDase-5	CD39L4 (possibly in macrophages)	28,31,34,35
NTPDase-6 (putative)	CD39L2 (protein not yet identified)	28,31,34

From Zimmermann[21], modified.

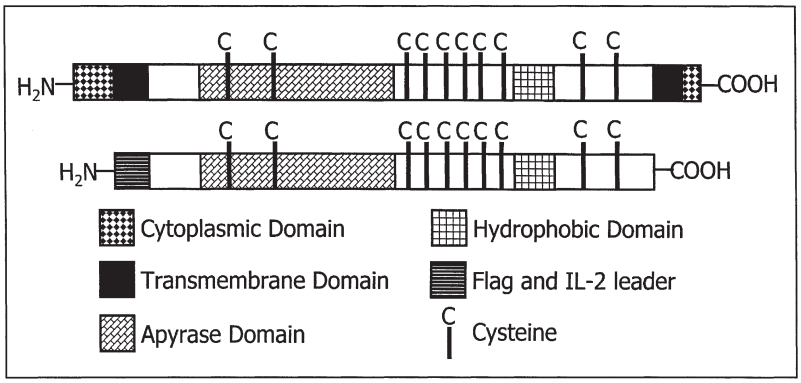

Figure 3. *Domain structure of ecto-ADPase/CD39. Two transmembrane regions are located near the amino and carboxyl termini; a hydrophobic sequence is centrally located. The putative apyrase conserved region is shown on the left side as apyrase domain, adjacent to the N-terminal portion. Cysteine residues are marked as C. An engineered form of soluble CD39, containing a flag tag and interleukin (IL)-2 secretion leader, and lacking the two transmembrane regions, is presented below for comparison.*

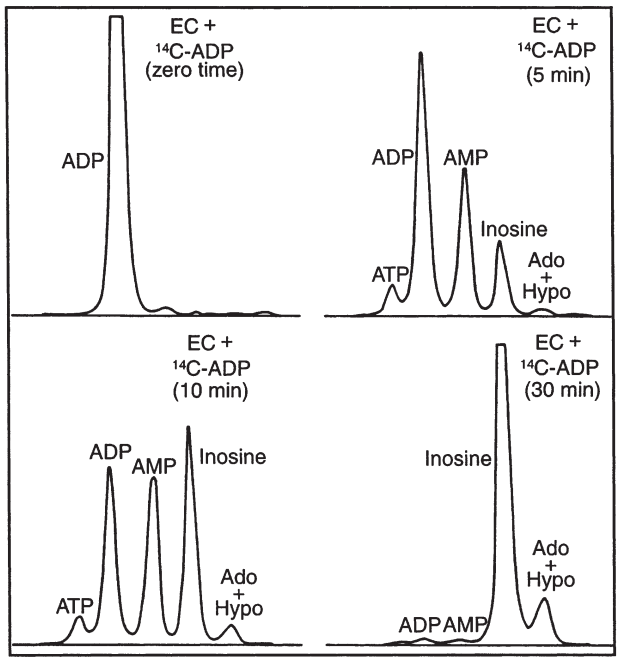

Figure 4. *Metabolism of ADP by EC. Human umbilical vein EC were incubated as indicated with 15 μM [14C]-ADP. ADP metabolites were separated by radio-thin layer chromatography. The activity of 5'-nucleotidase, as well as adenosine deaminase, is inferred from the rapid appearance of adenosine and final accumulation of inosine in these scans. Abbreviations as in figure 2.*

to inosine and hypoxanthine. Moreover, we found that supernatants from HUVEC incubated with [14C]-ADP could no longer induce aggregation in platelet-rich plasma, further indicating that ADP had been metabolized by endothelial ecto-nucleotidase[7]. Subsequently, we and others determined that the molecule responsible for this platelet inhibition was CD39[36,37]. Both abstracts appeared as full papers shortly thereafter[8,22].

Historically, Handa and Guidotti[38] had purified a soluble apyrase from potato tubers and cloned its cDNA. Sequence analysis revealed 25% amino acid identity and 48% amino acid homology with human CD39[38]. Maliszewski et al.[23] had cloned CD39 as a cell-surface glycoprotein expressed on activated B cells. Kansas et al.[39] had also shown that CD39 was present on natural killer cells and subsets of T cells as well as on preparations of HUVECs. Importantly, nucleotidases with homology to CD39 and potato apyrase are expressed extensively throughout the animal and vegetable kingdoms, in species such as the garden pea, *Caenorhabditis elegans* and *Toxoplasma*. There are at least four regions within the CD39 molecules which demonstrate extraordinary homology, and these are designated as apyrase conserved regions (ACR)[38].

Several additional experimental observations confirmed that the HUVEC ADPase is identical to CD39[8,22].

We were able to immunoprecipitate all ADPase activity from preparations of purified HUVEC membranes with several human CD39 antibodies. Confocal microscopy and indirect immunofluorescence studies demonstrated that CD39 is localized to the cell surface of HUVEC. COS cells, transfected with human or murine CD39 cDNA (but not vector alone), strongly inhibited ADP-induced platelet aggregation (Fig. 5)[23]. CD39-transfected COS cells metabolized ADP to AMP within 3 min, a time frame directly correlating with events leading to formation of a hemostatic plug or thrombus (data not shown). We noted that the time point at which platelet inhibition by CD39-expressing cells became evident was also within 3 min after ADP addition (Fig. 5)[23]. In fact, the time course for platelet inhibition by cells expressing CD39 correlates with their respective ADPase activities. These data demonstrated the importance of CD39 as a thromboregulator, and constitute strong evidence for a physiological function of CD39 as an ADPase. Thus CD39 may represent the culmination of an evolutionary process directed toward metabolizing prothrombotic platelet-derived nucleotides by an endothelial cell surface molecule, which controls excessive platelet accumulation and maintains blood fluidity.

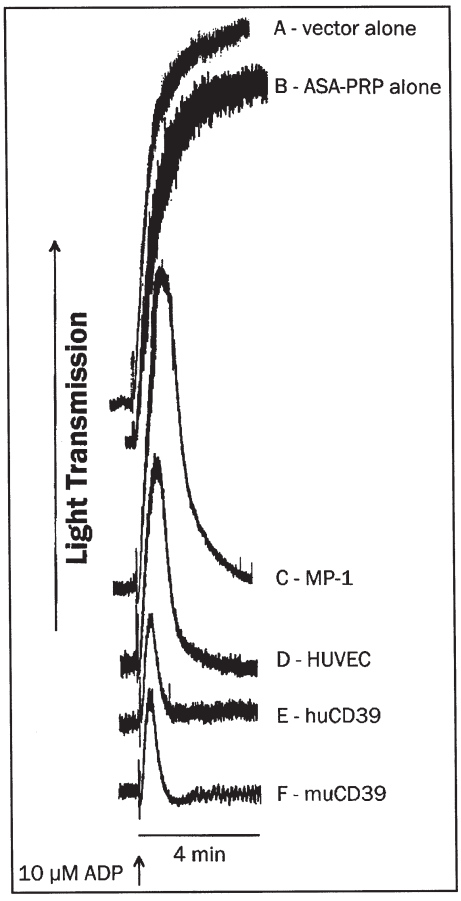

Figure 5. *Blockade and reversal of platelet aggregation to ADP by intact human umbilical vein endothelial cells (HUVEC), MP-1 cells (an activated B cell line[23]), and COS cells transfected with full-length human or murine CD39. Platelet-rich plasma (PRP) from a donor who had ingested aspirin (ASA) was stimulated with 10 µM ADP* (A) *in the presence of COS cells transfected with "empty" vector;* (B) *in the absence of any additions; and in the presence of* (C) *MP-1 cells,* (D) *HUVEC, and COS cells transfected with* (E) *human CD39 or* (F) *murine CD39. Expression of CD39 led to metabolism of the ADP component of the platelet releasate, and acquisition of platelet inhibitory activity due to blockade of platelet recruitment.*

Development of a recombinant soluble form of CD39/ecto-ADPase. As we studied the biochemical and biological properties of CD39, we began to realize that it could point to a novel strategy for blockade of platelet reactivity in platelet-driven occlusive vascular diseases. This premise was reinforced by our demonstration that the mode of action of CD39 is aspirin-independent, and that it blocks platelet reactivity even when eicosanoid formation and nitric oxide production are prevented. Importantly, the action of CD39 is not on the platelet *per se*; rather it metabolizes the ADP component of the activated platelet releasate, thus abolishing further platelet recruitment without any direct effect on the platelets themselves. This led to the hypothesis that a soluble form of human CD39 (solCD39) would constitute a novel systemic antithrombotic modality for treating thrombosis-prone patients with a low threshold for platelet activation. Subsequent experiments in porcine and murine models indicated that solCD39 could indeed efficiently inhibit platelet reactivity in the setting of acute, subacute, or chronic clinical situations. The design of solCD39 was based on the structure of full-length CD39 (Fig. 3), which contains two transmembrane regions near the amino and carboxyl termini, respectively. These domains serve to anchor the native protein in the cell membrane. Modeling studies, antibody epitope analyses and sequence homology have demonstrated that the portion of the molecule between the transmembrane regions is external to the cell[23]. The extracellular region contains the ACR characteristics of compounds in the apyrase family, in concordance with the hypothesis that the external portion of CD39 is critical for its ecto-ADPase activity. The ability of CD39-expressing cells to metabolize extracellular nucleotides supports the extracellular localization of the enzymatic portion of the molecule. The fact that intracellular nucleotide concentrations are maintained in the millimolar range further suggests that the active site of CD39 is not exposed to the cytoplasm.

In order to generate solCD39, the extracellular domain, encoding 439 amino acids, was isolated using oligonucleotide cassettes and polymerase chain reaction, and inserted into a mammalian expression vector. An N-terminal interleukin-2 leader sequence was added to ensure secretion of the recombinant molecule. Following transfection of COS cells with this solCD39-encoding plasmid, ATPase and ADPase activity in the conditioned medium increased linearly for a 5-day period. SolCD39 was then isolated from conditioned medium derived from transiently transfected COS cells via immunoaffinity chromatography,

using a CD39 monoclonal antibody. This resulted in a single ~66 kDa protein with both ATPase and ADPase activities, suggesting that the molecule was properly glycosylated by the COS cells. Upon removal of N-linked oligosaccharides by treatment with N-glycanase, SDS-PAGE analysis yielded a protein band with the predicted molecular weight of 52 kDa[40]. Purified solCD39 was subsequently tested for its effects on platelet aggregation *in vitro*. The molecule inhibits ADP, collagen, and thrombin receptor agonist peptide (TRAP)-induced platelet reactivity (Fig. 6). The inhibitory effects noted with collagen and TRAP suggested that these two agonists depend more on released ADP for aggregation and recruitment than we previously appreciated.

Site-directed mutagenesis studies of amino acids in the apyrase conserved regions of solCD39. To identify critically important amino acid residues for the enzymatic and biological activity of CD39, we performed site-directed mutagenesis studies within the highly conserved ACR of solCD39. Mutations of Glu 174 to Ala (E174A) and Ser 218 to Ala (S218A) resulted in loss of enzymatic activity. The ADPase activity of wild-type solCD39 and of each mutant was generally greater with calcium than with magnesium, but for ATPase activity, no such preference was observed. Y127A demonstrated the highest calcium/magnesium ADPase activity ratio, 2.8-fold higher than that of wild-type solCD39, but its enzyme activity was reduced by 50-60%. Importantly, in all cases enzymatic activity correlated precisely with biological activity in our platelet aggregation test system. For example, E174A, completely devoid of enzymatic activity, failed to inhibit platelet responsiveness. S218A, which lost 91% of its ADPase activity, was still capable of reversing platelet aggregation, albeit much less effectively than wild-type solCD39. We concluded that Glu 174 and Ser 218 were essential for both the enzymatic and biological activity of solCD39, whereas Tyr 127 played an important role as well[41].

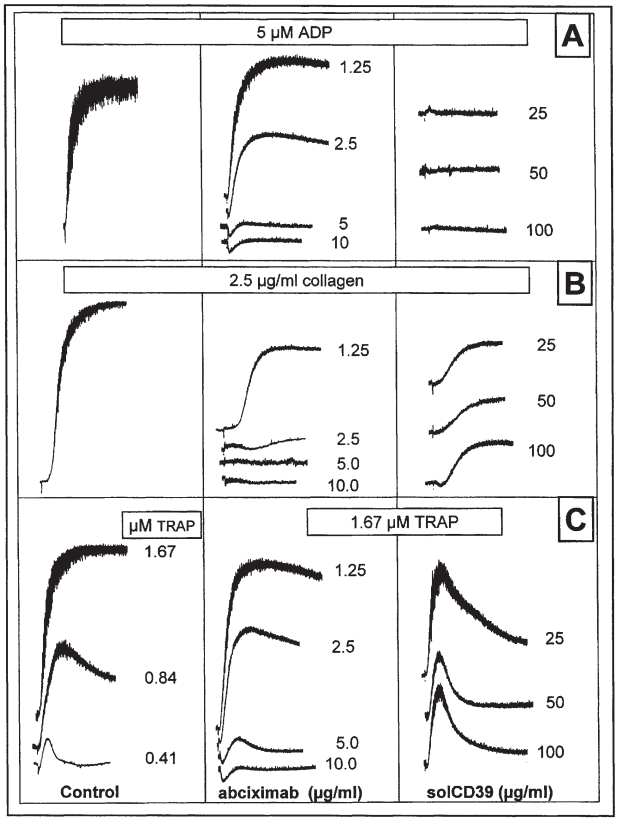

Figure 6. *Inhibition and reversal of platelet aggregation. Platelet-rich plasma from a donor who had ingested aspirin was stimulated with 5 µM ADP* (A)*, 2.5 µg/ml collagen (Chrono-Log)* (B)*, or thrombin receptor agonist peptide (TRAP)-6* (C) *as indicated.* In vitro *platelet responses to these agonists were strongly inhibited by both abciximab and a soluble form of human CD39 (solCD39).*

Summary and conclusions

Studies with a novel, soluble form of recombinant human ecto-ADPase, solCD39, indicate potential for a new class of antithrombotic agents acting by metabolism of ADP in an activated platelet releasate. Thus, solCD39 blocks and reverses platelet activation, preventing recruitment of additional platelets into a growing thrombus. The extent of occlusion and damage to the vascular wall associated with cardiac and cerebral vascular events such as stroke, myocardial infarction, angioplasty, and stenting can largely be attenuated. In addition, because of its independent mode of action on the platelet releasate, solCD39 could be administered in combination with currently utilized therapeutic modalities, including heparin, aspirin, and glycoprotein IIb/IIIa antagonists. We believe a new era in the therapeutics of vascular diseases is approaching.

References

1. Koch R. Über bakteriologische Forschung. Verhandlungen des X. Internationalen Medizinischen Congresses. (abstr) Berlin, 1891; 1: 35-47.
2. Ross R. Mechanisms of disease: atherosclerosis - an inflammatory disease. N Engl J Med 1999; 340: 115-26.
3. Libby P, Simon DI. Inflammation and thrombosis: the clot thickens. Circulation 2001; 103: 1718-20.
4. Marcus AJ, Broekman MJ, Drosopoulos JHF, et al. Thromboregulation by endothelial cells: significance for occlusive vascular diseases. Arterioscler Thromb Vasc Biol 2001; 21: 178-82.
5. Maclouf J, Murphy RC. Transcellular metabolism of neutrophil-derived leukotriene A_4 by human platelets. A potential cellular source of leukotriene C_4. J Biol Chem 1988; 263: 174-81.
6. Marcus AJ, Broekman MJ, Safier LB, et al. Formation of leukotrienes and other hydroxy acids during platelet-neutrophil interactions in vitro. Biochem Biophys Res Commun 1982; 109: 130-7.
7. Marcus AJ, Safier LB, Hajjar KA, et al. Inhibition of platelet function by an aspirin-insensitive endothelial cell

ADPase. Thromboregulation by endothelial cells. J Clin Invest 1991; 88: 1690-6.
8. Marcus AJ, Broekman MJ, Drosopoulos JHF, et al. The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39. J Clin Invest 1997; 99: 1351-60.
9. Marcus AJ, Weksler BB, Jaffe EA, Broekman MJ. Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. J Clin Invest 1980; 66: 979-86.
10. Schafer AI, Crawford DD, Gimbrone MA Jr. Unidirectional transfer of prostaglandin endoperoxides between platelets and endothelial cells. J Clin Invest 1984; 73: 1105-12.
11. Marcus AJ. Platelets: their role in hemostasis, thrombosis, and inflammation. In: Gallin JI, Snyderman R, eds. Inflammation: basic principles and clinical correlates. Philadelphia, PA: Lippincott, Williams & Wilkins, 1999: 77-95.
12. Marcus AJ, Safier LB. Thromboregulation: multicellular modulation of platelet reactivity in hemostasis and thrombosis. FASEB J 1993; 7: 516-22.
13. Serhan CN. Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? Prostaglandins 1997; 53: 107-37.
14. Marcus AJ, Safier LB, Ullman HL, et al. Platelet-neutrophil interactions. (12S)-hydroxyeicosatetraen-1,20-dioic acid: a new eicosanoid synthesized by unstimulated neutrophils from (12S)-20-dihydroxyeicosatetraenoic acid. J Biol Chem 1988; 263: 2223-9.
15. Marcus AJ, Hajjar DP. Vascular transcellular signalling. J Lipid Res 1993; 34: 2017-32.
16. Serhan CN, Haeggström JZ, Leslie CC. Lipid mediator networks in cell signaling: update and impact of cytokines. FASEB J 1996; 10: 1147-58.
17. Karim S, Habib A, Levy-Toledano S, Maclouf J. Cyclooxygenases-1 and -2 of endothelial cells utilize exogenous or endogenous arachidonic acid for transcellular production of thromboxane. J Biol Chem 1996; 271: 12042-8.
18. Ignarro LJ, Buga GM, Chaudhuri G. EDRF generation and release from perfused bovine pulmonary artery and vein. Eur J Pharmacol 1988; 149: 79-88.
19. Broekman MJ, Eiroa AM, Marcus AJ. Inhibition of human platelet reactivity by endothelium-derived relaxing factor from human umbilical vein endothelial cells in suspension. Blockade of aggregation and secretion by an aspirin-insensitive mechanism. Blood 1991; 78: 1033-40.
20. Plesner L. Ecto-ATPases: identities and functions. Int Rev Cytol 1995; 158: 141-214.
21. Zimmermann H. Extracellular metabolism of ATP and other nucleotides. Naunyn Schmiedebergs Arch Pharmacol 2000; 362: 299-309.
22. Kaczmarek E, Koziak K, Sévigny J, et al. Identification and characterization of CD39 vascular ATP diphosphohydrolase. J Biol Chem 1996; 271: 33116-22.
23. Maliszewski CR, Delespesse GJ, Schoenborn MA, et al. The CD39 lymphoid cell activation antigen. Molecular cloning and structural characterization. J Immunol 1994; 153: 3574-83.
24. Christoforidis S, Papamarcaki T, Galaris D, Kellner R, Tsolas O. Purification and properties of human placental ATP diphosphohydrolase. Eur J Biochem 1995; 234: 66-74.
25. Matsumoto M, Sakurai Y, Kokubo T, et al. The cDNA cloning of human placental ecto-ATP diphosphohydrolases I and II. FEBS Lett 1999; 453: 335-40.
26. Sévigny J, Picher M, Grondin G, Beaudoin AR. Purification and immunohistochemical localization of the ATP diphosphohydrolase in bovine lungs. Am J Physiol 1997; 272 (Part 1): L939-L950.
27. Wang TF, Guidotti G. CD39 is an ecto-(Ca^{2+}, Mg^{2+})-apyrase. J Biol Chem 1996; 271: 9898-901.
28. Chadwick BP, Frischauf AM. Cloning and mapping of a human and mouse gene with homology to ecto-ATPase genes. Mamm Genome 1997; 8: 668-72.
29. Kegel B, Braun N, Heine P, Maliszewski CR, Zimmermann H. An ecto-ATPase and an ecto-ATP diphosphohydrolase are expressed in rat brain. Neuropharmacology 1997; 36: 1189-200.
30. Kirley TL. Complementary DNA cloning and sequencing of the chicken muscle ecto-ATPase - Homology with the lymphoid cell activation antigen CD39. J Biol Chem 1997; 272: 1076-81.
31. Chadwick BP, Frischauf AM. The CD39-like gene family: identification of three new human members (CD39L2, CD39L3, and CD39L4), their murine homologues, and a member of the gene family from Drosophila melanogaster. Genomics 1998; 50: 357-67.
32. Smith TM, Kirley TL. Cloning, sequencing, and expression of a human brain ecto-apyrase related to both the ecto-ATPases and CD39 ecto-apyrases. Biochim Biophys Acta 1998; 1386: 65-78.
33. Wang TF, Guidotti G. Golgi localization and functional expression of human uridine diphosphatase. J Biol Chem 1998; 273: 11392-9.
34. Chadwick BP, Williamson J, Sheer D, Frischauf AM. cDNA cloning and chromosomal mapping of a mouse gene with homology to NTPases. Mamm Genome 1998; 9: 162-4.
35. Mulero JJ, Yeung G, Nelken ST, Ford JE. CD39-L4 is a secreted human apyrase, specific for the hydrolysis of nucleoside diphosphates. J Biol Chem 1999; 274: 20064-7.
36. Marcus AJ, Broekman MJ, Drosopoulos JHF, et al. CD39 is the endothelial cell ecto-ADPase responsible for inhibition of platelet function. (abstr) Blood 1996; 88: 465A.
37. Koziak K, Kaczmarek E, Siegel JB, Bach FH, Robson SC. CD39 expressed by endothelial cells, platelets and monocytes has ATPDase activity. (abstr) Blood 1996; 88: 465A.
38. Handa M, Guidotti G. Purification and cloning of a soluble ATP-diphosphohydrolase (apyrase) from potato tubers (Solanum tuberosum). Biochem Biophys Res Commun 1996; 218: 916-23.
39. Kansas GS, Wood GS, Tedder TF. Expression, distribution, and biochemistry of human CD39. Role in activation-associated homotypic adhesion of lymphocytes. J Immunol 1991; 146: 2235-44.
40. Gayle RB III, Maliszewski CR, Gimpel SD, et al. Inhibition of platelet function by recombinant soluble ecto-ADPase/CD39. J Clin Invest 1998; 101: 1851-9.
41. Drosopoulos JHF, Broekman MJ, Islam N, Maliszewski CR, Gayle RB III, Marcus AJ. Site-directed mutagenesis of human endothelial cell ecto-ADPase/soluble CD39. Requirement of glutamate174 and serine218 for enzyme activity and inhibition of platelet recruitment. Biochemistry 2000; 39: 6936-43.